The Dynamic Regulation of GABAergic Synapses Affects Cell Growth and Structural Plasticity of Adult-born Neurons

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ZUSAMMENFASSUNG

GABA ist der wichtigste inhibitorische Neurotransmitter im adulten ZNS, der die neuronale Erregbarkeit, die Feuer-Eigenschaften der Nervenzellen und die Synchronisation der Netzwerkaktivitäten reguliert. Jüngste Studien zeigen, dass GABAerge Synapsen durch ihre dynamischen Eigenschaften die kurzfristige Verstärkung und Abschwächung von GABAergen Signalen erlauben, und so das Gleichgewicht von Erregung und Hemmung nach Änderungen in der Netzwerkaktivität aufrechterhalten. Gephyrin, das Hauptgerüstprotein der GABAergen postsynaptischen Dichte (PSD), stellt ein zentrales Element in der synaptischen GABAergen Plastizität dar. *In vitro*-Studien haben gezeigt, dass Gephyrin die Bildung und Aufrechterhaltung der GABAergen Synapsen reguliert, indem seine Gerüsteigenschaften durch post-translationale Modifikationen angepasst werden. Weiterhin interagiert Gephyrin an der GABAergen PSD mit mehreren nachgeschalteten Signalmolekülen, die ihrerseits mit dem Zytoskelett interagieren und somit zu funktionellen und strukturellen Anpassungen an der Synapse beitragen.

GABAerge Signale regulieren unterschiedliche Schritte in der Neurogenese des sich entwickelnden Gehirns und des adulten Gehirns. Daher sollte die Relevanz der GABAergen synaptischen Plastizität für die korrekte neuronale Differenzierung und synaptische Integration im Rahmen dieser Arbeit im Tiermodell in vivo untersucht werden.

In einer ersten Studie wurde der Einfluss von postsynaptischen Gephyrin-Clustern auf die Regulierung der Signalwege untersucht, welche die Entwicklung von adult-geborenen Neuronen im Riechkolben beeinflussen. Zu diesem Zweck wurden Gephyrin und Gephyrinmutanten, welche am N-Terminus mit eGFP fusioniert sind, mithilfe von Lentiviren in adult-geborenen Körnerzellen überexprimiert, um so die Effekte eines phospho-defizienten eGFP-Geph(S270A) Konstrukts (begünstigt in vitro die GABAerge Synapsenbildung) und eines dominant-negativen Konstrukts, eGFP-Geph(L2B) (reduziert die Clusterbildung von Gephyrin) vergleichend näher zu untersuchen. Die morphologische Entwicklung der transfizierten Körnerzellen wurde durch immunhistochemische Färbungen und Licht-, bzw. Elektronenmikroskopie verfolgt, wobei eGFP-Geph und eGFP als Kontrollen dienten.

Die Überexpression von eGFP-Geph(S270A) führt zu einer stärkeren Verzweigung der Dendriten und der Bildung von transienten GABAergen Dornensynapsen, welche α2-$GABA_A$ -Rezeptoren beherbergen, wohingegen, die Überexpression von eGFP-Geph(L2B), das Wachstum und das Verzweigen der Dendriten sowie die Bildung von Dornensynapsen (und damit deren synaptischen Input) stark beeinträchtigt. Darüber hinaus wurde die Lebensdauer der eGFP-

Geph(L2B) exprimierenden Körnerzellen verkürzt, während die eGFP-Geph(S270A) exprimierenden Körnerzellen eine normale Lebensdauer aufwiesen. Diese Resultate zeigen, dass die Clusterbildung von postsynaptischem Gephyrin und dessen Regulation durch phosphorylierungsabhängige Mechanismen, essentielle Eigenschaften sind, die die Entwicklung von adult-geborenen Körnerzellen steuern. Sehr wahrscheinlich geschieht dies durch Veränderungen der Funktion und Lokalisierung der Signalmoleküle, welche mit dem Gephyringerüst interagieren, Daher dient das postsynaptische Gerüst, das von Gephyrin organisiert wird, als Signalstelle, welche nicht nur die GABAerge Synapsenfunktion und deren Plastizität regelt, sondern auch die Reifung von Neuronen.

In einer zweiten Studie wurde die Funktion des α5-$GABA_A$ Rezeptors in der Ent-wicklung von adult-geborenen Körnerzellen im Gyrus dentatus untersucht. Diese Rezeptoren vermitteln hauptsächlich eine tonische Inhibition in der hippocampalen Formation. Aus Erklärungsmodellen von kognitiven Störungen ist bekannt, dass eine Verminderung ihrer Funktion zu verbesserten Lern- und Gedächtnisfähigkeiten führt. Um die Funktion des α5-$GABA_A$ Rezeptors in diesem Zusammenhang besser zu verstehen, wurden adult-geborenen Körnerzellen im Gyrus dentatus von Wildtyp-Kontroll-, α5-KO und α5-heterozygoten Mäusen, mittels eGFP kodierender Retroviren markiert und somit geburtsdatiert. Dabei verursachte die globale Deletion von *Gabra5* keine offensichtlichen morphologischen Veränderungen in den adult-geborenen Körnerzellen, wohingegen, die Expression nur einer Kopie des α5-$GABA_A$ Rezeptors die Migrationsdistanz, das dendritische Wachstum und die Verzweigung der Körnerzellen reduzierte. Grösstenteils waren diese Effekte zellautonom, wie durch die Cre-vermittelte Deletion der α5 Untereinheit selektiv in adult-geborenen Körnerzellen von Mäusen, die gefloxte *Gabra5* Allele aufweisen, gezeigt werden konnte. Diese Beobachtungen führen zu dem Schluss, dass eine reduzierte Signalweiterleitung durch α5-$GABA_A$ Rezeptoren oder nachgeschaltete Signalwege, einen dauerhaften Entwicklungsrückstand in der Reifung von adult-geborenen Körnerzellen verursachen kann.

Zusammenfassend lassen die Ergebnisse der im Rahmen dieser Arbeit durchgeführten Studien vermuten, dass die GABAerge Signalweiterleitung eng mit der Aktivität der Signalkaskaden verknüpft ist, die zur Regulierung der dynamischen Vorgänge im Zytoskelett beitragen.
Die Aktivierung der nachgeschalteten Signalmoleküle kann entweder durch Interaktion mit Gephyrin an der GABAergen PSD stattfinden oder durch die Änderung neuronaler Erregbarkeit,

die durch die extrasynaptisch lokalisierten $GABA_A$ Rezeptoren verursacht wird. Die Mechanismen, welche für die Reifung und funktionelle Integration von adult-geborenen Neuronen von Bedeutung sind, könnten in der Pathophysiologie neurologischer und psychiatrischer Entwicklungskrankheiten eine relevante Rolle spielen und eventuell neue potentielle Wege für therapeutische Eingriffe eröffnen.

ABSTRACT

GABA is the main inhibitory neurotransmitter in the adult CNS, regulating neuronal excitability, firing properties, and synchronization of network activity. Recent studies have revealed that GABAergic synapses exhibit dynamic properties that allow strengthening or weakening GABAergic transmission on a short time-scale to maintain the balance of excitation and inhibition upon alterations in network activity. Gephyrin, the main scaffolding protein of the GABAergic postsynaptic density (PSD), is a central element of GABAergic synaptic plasticity. *In vitro* work has demonstrated that gephyrin regulates the formation and maintenance of GABAergic synapses by adjusting its scaffolding properties through post-translational modifications. Further, gephyrin interacts with several down-stream signaling molecules at the GABAergic PSD, which interact with the actin cytoskeleton and contribute to functional and structural adaptations at the synapse.

In the present PhD thesis, I took advantage of the fact that GABAergic signaling modulates specific steps of neurogenesis in developing and adult brain to test *in vivo* the relevance of GABAergic synapse plasticity for proper neuronal differentiation and synaptic integration.

In a first study, I investigated the impact of gephyrin postsynaptic clustering for regulating signaling pathways that control the development of adult-born neurons in the olfactory bulb. To this end, we used lentivirus-mediated over-expression of gephyrin and gephyrin mutants, N-terminally fused to eGFP, in adult-born GCs. We compared the effects of a phospho-deficient eGFP-Geph(S270A) construct, which favors GABAergic synapse formation *in vitro* and of a dominant-negative construct, eGFP-Geph(L2B), which prevents gephyrin clustering. Morphological development of transfected GCs was monitored by immunohistochemical staining for light- and electron-microscopy, using eGFP-Geph and eGFP alone as controls.

Over-expression of eGFP-Geph(S270A) favored dendritic branching and the formation of transient GABAergic synapses on spines, containing α2-$GABA_A$Rs. In contrast, over-expression of eGFP-Geph(L2B) strongly impaired dendritic growth and branching, as well as the formation of spines, and thereby synaptic inputs. In addition, long-term survival of GCs expressing eGFP-Geph(L2B) was curtailed, whereas GCs expressing eGFP-Geph(S270A) had a normal life-span. These results show that formation of postsynaptic gephyrin clusters, and their regulation by phosphorylation-dependent mechanisms, are essential features governing the development of adult-born GCs, most likely by modulating the function or localization of signaling molecules that interact with the gephyrin scaffold. Therefore, the postsynaptic scaffolds organized by

gephyrin serve as signaling hubs regulating not only GABAergic synapse function and plasticity, but also neuronal maturation.

In the second study, I investigated the role of α5-$GABA_A$Rs for the development of adult-born dentate gyrus GCs. These receptors mediate mainly tonic inhibition in the hippocampal formation, and a reduction in their function is known to improve learning and memory function in models of cognitive disability. For the purpose of our work, we birth-dated and labeled adult-born GCs using retroviruses encoding eGFP, injected in the hilus of the dentate gyrus of wild-type control, α5-KO, and α5-heterozygous (α5-het) mice. Unexpectedly, the global deletion of *Gabra5* caused no obvious morphological alterations in adult-born GCs. In contrast, a partial reduction of α5-$GABA_A$Rs in α5-het mice affected their migration, as well as dendritic growth and arborization. To a large extent, these effects were cell-autonomous, as verified by Cre-mediated deletion of the α5 subunit selectively in adult-born GCs of mice carrying floxed *Gabra5* alleles. Taken together, these observations reveal that impaired α5-$GABA_A$R-mediated transmission or down-stream signaling causes enduring deficits in the maturation of adult-born GCs.

In conclusion, the results of these two studies suggest that GABAergic transmission is linked to the activity of signaling cascades, which contribute to the regulation of cytoskeleton dynamics. This activation of down-stream signaling molecules can occur either via interactions with the gephyrin scaffold at GABAergic PSDs or by altering neuronal excitability regulated by extra-synaptic $GABA_A$Rs. The major role played by these mechanisms for the maturation and functional integration of adult-born neurons might be relevant for the pathophysiology of neurodevelopmental neurological and psychiatric diseases and opens new potential avenues for therapeutic intervention.

I. GENERAL INTRODUCTION

1 GABAergic transmission

y-aminobutyric acid (GABA), synthesized by decarboxylation of glutamate, is one of the most important mediators of inhibitory neurotransmission in the adult mammalian central nervous system (CNS). During brain development, GABA mediates excitatory signals and acts as a trophic factor regulating proliferation and differentiation of neuronal progenitors, neuronal migration, dendritic maturation and synapse formation (Represa and Ben-Ari, 2005, Wang and Kriegstein, 2009). One of the major roles of GABA in the adult CNS is to keep the balance between excitation and inhibition (E/I) in neuronal circuits and to synchronize neuronal network oscillations. An imbalance due to abnormal GABAergic signaling has been implicated in various diseases, such as epilepsy, autism, depression, anxiety, and schizophrenia.

GABA acting as a neurotransmitter can bind either fast responding ionotropic receptors (the ligand-gated, chloride-permeable $GABA_A$ and $GABA_C$ receptors) or slow responding metabotropic receptors ($GABA_B$) that are negatively coupled to adenylyl cyclase. Both types of receptors are expressed in the developing and adult CNS, where they play fundamentally different roles, as pointed out above.

1.1 Interneurons

In the mammalian CNS, interneurons are typically inhibitory neurons, using mainly the neurotransmitter GABA. Inhibitory interneurons are important to control excitatory forces of large groups of principal cells and thereby they generate and synchronize neuronal oscillations. Synchronized oscillatory activity of neuronal ensembles has been described to be important in information processing, memory formation (learning and retrieval), conscious perception, sensory perception and motor control, and therefore it occurs in many different region of the CNS (Varela et al., 2001). There is a remarkable cellular diversity of interneurons, which are classified according to their temporal firing patterns, molecular composition and innervations of distinct subcellular domains of principal cells (Klausberger, 2009). For example, there are at least 21 classes of interneurons in the hippocampus, and in the olfactory bulb (OB) there are different subtypes of granule cells (GC), periglomerular cells (PGC) and short axon cells (Lagier et al., 2004, Klausberger and Somogyi, 2008, Lepousez et al., 2013).

The generation of neural oscillations can occur in three different ways, feed-forward and feedback inhibition, mostly found in the hippocampus, and lateral inhibition, found in the OB. Through feed-forward inhibition, one population of neurons gets silenced from the activity of another one, whereas feedback inhibition allows the most active principal cell to limit the activity of all other less active cells. In the OB, lateral inhibition enables to generate γ-oscillations by dendro-dendritic reciprocal synapses between GCs and their principal cells. In the hippocampus oscillations with various frequencies occur which correlate to behavioral state: theta oscillations (4-12 Hz) are generated during navigation, learning, memory formation and retrieval; ripple oscillations (120-200 Hz) occur during resting behavior; γ-oscillations (30-80 Hz) modulate theta activity and are thought to rely on temporal coding (Klausberger, 2009). Not only the type of interneuron, but also the functional and molecular properties of principal cells, can influence network oscillations (Kasugai et al., 2010).

Beside the distinct functional properties of interneurons and principal cells in the cerebral cortex, they are generated in different proliferative areas of the brain during embryonic development (Wilson and Rubenstein, 2000, Kriegstein and Noctor, 2004). Whereas principal cells are born in the embryonic pallium of the dorsal telencephalon and migrate radially to their final position, interneurons are primarily generated in the subpallium of the ventral telencephalon. The subpallium consists of the embryonic ganglionic eminence which can be subdivided into the medial, lateral and caudal ganglionic eminences. The majorities of the GABAergic interneurons are born in the medial and caudal ganglionic eminences and generally follow tangential migration to their final location in the cerebral cortex (reviewed in (Wilson and Rubenstein, 2000, Kelsom and Lu, 2013)).

1.2 GABAergic synapse

In the nervous system, communication between individual neurons is ensured through synapses. In 1959, after the invention of electron microscopy technique, George Gray provided the first clear evidence of synaptic contacts. He described two types of synapses (based on the membrane thickening of the postsynapse), type I and type II. The former are asymmetric synapses and found on dendritic spines and shafts, later identified to be excitatory synapses. Whereas the latter, type 2 characterized as symmetric, are inhibitory synapses and located primarily on dendritic shafts and cell bodies (Guillery, 2000). Inhibitory neurotransmission in the CNS is mainly mediated through GABA, released from the presynaptic site of inhibitory neurons and $GABA_A$ receptors ($GABA_AR$) located at the postsynaptic site.

In the next section, I describe first the molecular organization of GABA receptors, emphasizing the $GABA_ARs$, and then the molecular organization and plasticity of GABAergic synapses with the focus on its postsynaptic scaffold protein gephyrin.

1.3 Molecular organization of $GABA_A$ receptors

The heteropentameric $GABA_ARs$ are members of the Cys-loop ligand-gated ion channel family, which also include the nicotinic acetylcholine receptors, glycine receptors (GlyR), and serotonin type 3 receptors (Olsen and Sieghart, 2009). These receptors have all a C- and N-terminal domain located extracellularly and four transmembrane (TM1-4) domains with a large cytoplasmic loop between TM3 and TM4, allowing interactions with postsynaptic scaffold structures, signaling molecules, and trafficking proteins. $GABA_AR$ subunits are phospho-proteins, forming a pentameric assembly selectively permeable for Cl^- and bicarbonate ions. $GABA_ARs$ cause shunting inhibition and mediate depolarization or hyperpolarization, depending on the reversal Cl^- potential. In immature cells, $GABA_AR$ activation is depolarizing, owing to a high $[Cl]_i$, whereas in mature neurons, $GABA_AR$ activation leads to a hyperpolarization due to a decrease in $[Cl]_i$. This timely regulated switch from depolarizing to hyperpolarizing depends on the relative expression and function of Na^+-K^+-Cl^- co-transporter type 1 (NKCC1) and the K^+-Cl^- co transporter type 2 (KCC2),which mediate Cl^- fluxes in opposite directions.

$GABA_ARs$ are encoded by a family of 16 subunit genes divided in subgroups based on sequence homology: α (1-6), β (1-3), γ (1-3), δ, ε and θ (Macdonald and Olsen, 1994, Sieghart et al., 1999, Sieghart and Sperk, 2002). Different subunit compositions give rise to distinct $GABA_AR$ subtypes and determine their functional properties and localization throughout the brain. In the CNS, the majority of fast, synaptic GABAergic transmission is mediated through $GABA_AR$-subtypes with the stoichiometries of two α1, α2, α3 subunits together with two β2 or β3 and one γ2 subunit, the latter is known to be crucial for the anchoring at the synapse (Essrich et al., 1998, Rudolph and Mohler, 2004, Tretter et al., 2012). In addition, these $GABA_AR$ subtypes are mostly anchored by gephyrin, the main scaffolding protein of inhibitory synapses, at the plasma membrane of the postsynaptic density (PSD). In contrast, receptors containing the α4-6, β,δ or γ2 subunits appear extrasynaptically and mediate tonic (slow) GABAergic transmission through ambient GABA (μM range) (Nusser et al., 1998, Chandra et al., 2006, Luscher et al., 2011a). Radixin, an α5 subunit-interacting protein, was identified to be essential for the clustering of α5-$GABA_ARs$ at the plasma membrane (Loebrich et al., 2006). This knowledge reveals that the

localization of $GABA_A$Rs in the membrane depends on the α-subunit variants. In addition, analysis of α-subunit null mice revealed the essential role of the α-subunit for the assembly and cell surface expression of the entire receptor complex (Studler et al., 2005, Kralic et al., 2006, Patrizi et al., 2008, Duveau et al., 2011, Panzanelli et al., 2011, Pallotto et al., 2012).

This heterogeneity of $GABA_A$Rs reflects the differences in subunit expression patterns during development and in the adult brain, as well as their different functional and pharmacological properties. Immunohistochemistry analysis revealed different molecular structures and cellular localization of $GABA_A$R in the neonatal and mature brain. In particular, the α5-containing $GABA_A$Rs are strongly expressed prenatally, but decreases with aging brain, while other subunits of the adult brain (α4, α6, δ) are sparsely expressed or even absent in the neonatal brain. In contrast, the expression of α1-containing $GABA_A$Rs increases during postnatal development throughout most brain regions, whereas the expression of α2 subunit get restricted to specific brain areas in the mature brain (Fritschy et al., 1994, Paysan et al., 1997, Ramos et al., 2004).

Beside the function as an important neurotransmitter in the brain, GABA respectively $GABA_A$Rs containing the α1-, α2-, α3-, α5-subunit are also molecular targets for benzodiazepines (BZ) which have a sedative, anticonvulsant and anxiolytic effect (Rudolph et al., 1999, Rudolph and Mohler, 2014). By binding the interface between the α- and γ-subunit of the $GABA_A$R, BZs potentiate GABAergic transmission by enhancing the channel-opening frequency through a conformational change leading to a higher affinity of GABA to the receptor. In contrast, barbiturates and neurosteroids act as potent positive allosteric modulators of the $GABA_A$R, by enhancing the channel opening time (Mitchell et al., 2008, Tan et al., 2011).

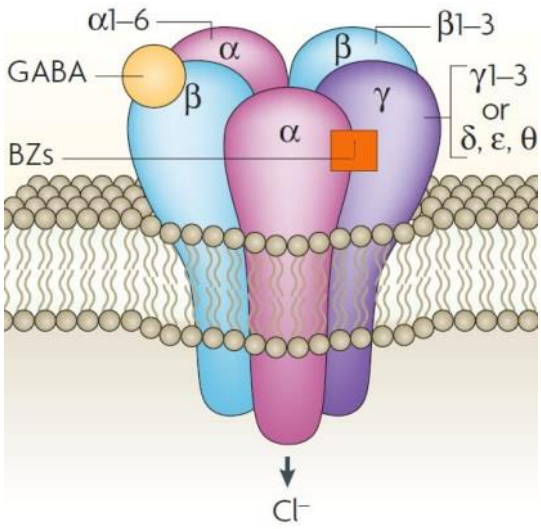

Figure 1 Pentameric assembly of $GABA_AR$ subunits
Five subunits from different subfamilies (α, β, γ, δ, ε, and θ) form a heteropentameric Cl^--permeable channel. The majority of $GABA_ARs$ expressed in the brain consist two α, two β, and one γ subunits. The binding of the neurotransmitter GABA occurs at the interface between the α and β subunits and leads to the opening of the channel and thereby Cl^--influx. Benzodiazepine site ligands bind the interface between the α and γ subunits and allosterically modulate GABAergic transmission (Jacob et al., 2008).

1.4 Molecular organization and plasticity of GABAergic synapses

GABAergic neurotransmission needs to be precisely regulated and coordinated at the postsynaptic site. Clustering of $GABA_ARs$ at the membrane and direct or indirect interactions with their associated proteins of PSD, is essential to maintain proper synaptic transmission. The presence of functional $GABA_ARs$ at the plasma membrane is subjected to continuous replacement with synaptic located receptors and receptors located extrasynaptically in a reserve pool (Thomas et al., 2005).

The PSD of inhibitory synapses typically harbors $GABA_ARs$ (or GlyRs) and many signaling proteins: in particular, cell adhesion molecules, such as neuroligin-2 (NL2), which enable the contact with the presynaptic terminal; cytoskeletal associated proteins, including gephyrin, collybistin (CB), Mena/VASP, dystrophin dystroglycan complex which are necessary for the stabilization and plasticity of the post-synapse; and motor proteins, such as KIF5 contributing to cargo transport. The interaction between these proteins and the $GABA_ARs$ enables the synapse to respond fast upon changes in network activity, by structural and functional adaption.

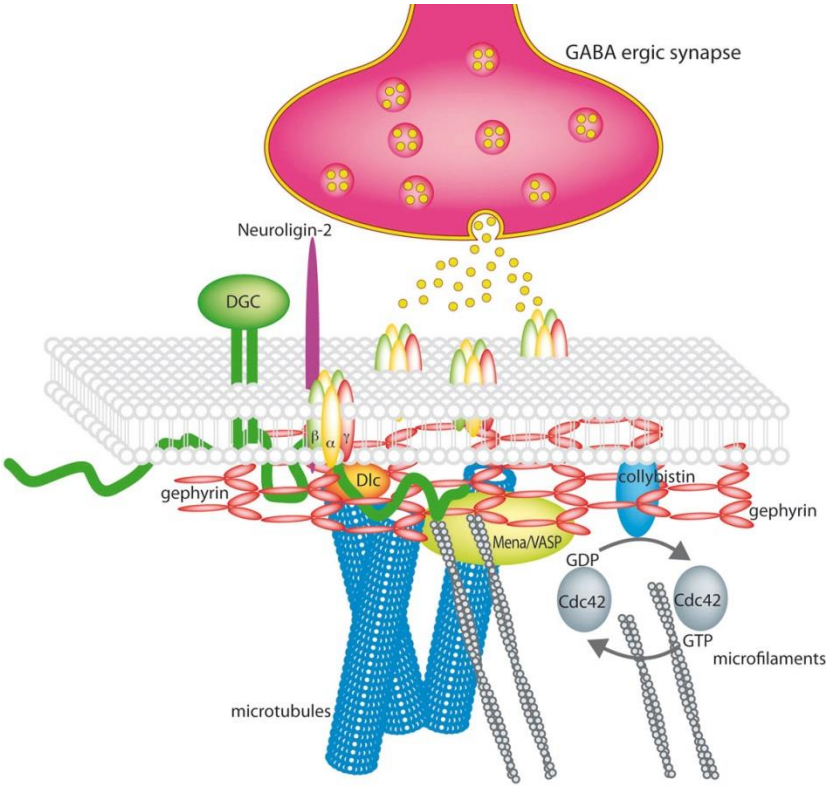

Figure 2 Organization of GABAergic synapse
Synaptic $GABA_AR$s are stabilized via gephyrin scaffold at the postsynaptic membrane. Collybistin interacts with gephyrin and activated Cdc42 which initiates local remodeling of the cytoskeleton. Dlc1/2 and Mena/VASP are cytoskeleton-associated protein. Neuroligin-2 is a cell adhesion molecule, which enables the contact with the presynaptic terminal. The dystrophin-glycoprotein complex (DGC) stabilizes the synapse (Tretter and Moss, 2008).

I focus on gephyrin, the main scaffolding protein in the inhibitory PSD and known to be the most important protein for $GABA_AR$ stabilization at the synapse, but I also introduce additionally two important components of the PSD.

Neuroligin

NLs are cell adhesion proteins anchored at the postsynaptic membrane by interacting with the presynaptically located neurexins. Neurexins are as well adhesion molecules and found in the active zone of the presynaptic membranes, where they contribute to the localization of synaptic vesicles, docking proteins and fusion molecules. The interaction between NLs and neurexins is sufficient for synapse formation, leading to a recruitment and stabilization of other synaptic proteins (Scheiffele et al., 2000, Graf et al., 2004, Dong et al., 2007). There are four different neuroligin (NL1-4) isoforms, whereby NL1 is selectively found at glutamatergic synapses, NL2 at GABAergic synapses, NL4 at glycinergic synapses and NL3 is located at glutamatergic and

GABAergic ones (Varoqueaux et al., 2004, Budreck and Scheiffele, 2007, Baudouin and Scheiffele, 2010, Hoon et al., 2011).

The interaction of NL2 with its extracellular site to specific neurexin variants, and with its intracellular domain to gephyrin, is required for structural and functional maturation of subsets of GABAergic synapses. However, these proteins have to form a complex with CB to be sufficient to form a scaffold at the GABAergic synapse (Varoqueaux et al., 2006, Gibson et al., 2009, Hoon et al., 2009, Poulopoulos et al., 2009).

Mutation or functional abnormalities both in NL and neurexin can cause neurodevelopmental disorders, schizophrenia and autism-spectrum disorders, known from human patients (Sudhof, 2008, Bourgeron, 2009).

Collybistin

CB is a neuron-specific protein and acts as a guanine nucleotide exchange factor (GEF) onto small Rho GTPases Cdc42 and TC-10 in the CNS (Mayer et al., 2013). There are three known CB isoforms, arising from alternative splicing of *ARHGEF9*, each one either containing or lacking an N-terminal SH3 domain ($CB1_{SH3+/-}$, $CB2_{SH3+/-}$, $CB3_{SH3+/-}$) (Harvey et al., 2004). CB is identified as a protein which binds gephyrin, in order to regulate gephyrin clustering at postsynaptic site (Kins et al., 2000, Saiepour et al., 2010, Tyagarajan et al., 2011a). In the hippocampus of CB-deficient mice (*Arhgef9*-null), a decrease in GABAergic transmission is measurable accompanied with a loss of gephyrin and gephyrin-dependent $GABA_A$Rs at postsynaptic sites, whereas CB is not required for the localization of GlyRs at postsynaptic sites (Papadopoulos et al., 2007). Likewise, CB is able to interact with NL2 at GABAergic synapses, via the SH3+ isoforms (Poulopoulos et al., 2009). The fact that CB activates Cdc42, which initiates local remodeling of the actin filaments, suggests that this interaction might regulate gephyrin scaffold through the rearrangement of the cytoskeleton (Xiang et al., 2006).

In patients with X-linked mental retardation, provoked by a mutation in the ARHGEF9 gene, the importance of the NL2-gephyrin-CB complex becomes clear (Harvey et al., 2004). However, the exact function of CB, with its underlying mechanisms, requiring different splicing isoforms, remains unclear.

Gephyrin

Gephyrin, a 93 kDa multifunctional cytoplasmic protein, is evolutionarily highly conserved and is required for the biosynthesis of molybdenum cofactor (Moco) in non-neuronal cells (Feng et al., 1998, Schwarz et al., 2009). In addition, in neurons, gephyrin is the core scaffolding protein of the PSD of GABAergic and glycinergic synapses, allowing the regulation of $GABA_AR$ and GlyR clustering and its stabilization by forming a scaffold anchored to the cytoskeleton (Pfeiffer et al., 1982, Belaidi and Schwarz, 2013). Gephyrin was identified in affinity-purified GlyR preparations of the spinal cord, where it anchors the receptor to the cytoskeleton (Kirsch et al., 1991). Therefore, this polypeptide was named from the Greek word for bridge (gejura). In 1995 Sassoè-Pognetto et al. demonstrated the presence of gephyrin in identified GABAergic synapses of the retina (Sassoe-Pognetto et al., 1995, Sassoe-Pognetto et al., 2000, Fritschy et al., 2008). Whereas gephyrin is essential for GlyR clustering at the plasma membrane, only a subset of $GABA_AR$ subtypes, mainly those containing the α1-, α2-, α3-subunit, interact with gephyrin, albeit with a 500-fold lower binding-affinity compared to the GlyR-gephyrin interaction (Kneussel et al., 2001, Levi et al., 2004, Maric et al., 2011). The absence of gephyrin results in the loss of $GABA_ARs$ at the synapse (Essrich et al., 1998, Kneussel et al., 1999). In turn, gephyrin depends on the presence of postsynaptic $GABA_ARs$ to form a cluster within the PSD of GABAergic synapses (Kralic et al., 2006, Patrizi et al., 2008, Panzanelli et al., 2011, Pallotto et al., 2012). Gephyrin consists of three functional domains, the G domain in the N-terminal, the E domain in the C-terminal and a large C-linker domain. The sequences of the G and E domains exhibit similarities with Moco-synthesizing enzymes MogA and MoeA in bacteria and plants (Liu et al., 2000, Sola et al., 2001, Sola et al., 2004). Gephyrin has the ability to self-multimerize and form a presumably hexagonal scaffolding lattice, through its G and E domain, whereas the clustering function is regulated by the E domain (Lardi-Studler et al., 2007, Herweg and Schwarz, 2012, Tretter et al., 2012). It was shown that a mutation in the E domain of rat gephyrin by replacing 8 out of 10 residues (DIDGVRKI) forming a surface-exposed loop (L2B) with their homologues from bacterial MoeA (KL..SNSW) prevents gephyrin from clustering, accompanied with a failure in formation of α2-$GABA_ARs$ at the membrane. In addition, a complete lack of the E domain induces the same phenotype (Lardi-Studler et al., 2007). In contrast, replacing only 6 residues (IDGVRK) by their homologues (L..SNS) strongly increases the aggregation propensity of gephyrin, leading to formation of supernumerary postsynaptic clusters (Lardi-Studler et al., 2007) However, the formation of postsynaptic gephyrin clusters

depends as well on the presence of postsynaptic $GABA_A$Rs (Essrich et al., 1998, Yu et al., 2007, Panzanelli et al., 2011, Pallotto et al., 2012).

The solvent-exposed C domain contains target sites for a number of gephyrin-interacting proteins, further it is also sensitive to proteolytic degradation (Fritschy et al., 2008).

The mechanisms by which gephyrin anchors $GABA_A$Rs at the postsynaptic site and interacts with several signaling molecules and therefore determines the strength of the GABAergic transmission is poorly understood.

1.5 Post-translational modification of gephyrin

A correct E/I-balance upon activity-dependent changes in neuronal circuits depends on fast molecular and structural modification of synapses. One way for regulating the GABAergic transmission after changes in activity, is to modulate $GABA_A$R channel gating properties, or the functional behavior of proteins in the PSD. Post-translational modifications, such as phosphorylation, acetylation and palmitoylation, are common mechanisms for adjusting the properties of proteins.

It is known that post-translational modification of gephyrin has a great impact on its functions, scaffolding structure and localization at the postsynaptic site. By mass-spectrometry multiple phosphorylation residues on gephyrin were identified, which are targets for serine/threonine kinases, which might modulate GABAergic transmission by affecting synaptic function and homeostasis (Tyagarajan et al., 2011b, Herweg and Schwarz, 2012, Tyagarajan et al., 2013).

In particular, Serine 270 (Ser270) residue was identified to be a phosphorylation site targeted by glycogen synthase kinase 3β (GSK3β), a proline-directed kinase, to regulate GABAergic transmission (Tyagarajan et al., 2011b). Abolishing the phosphorylation of Ser270 using site directed mutagenesis (S270A) or by pharmacological inhibition of GSK3β favors the formation of supernumerary clusters of $GABA_A$Rs containing the α2 subunit in cultured hippocampal neurons. In contrast, phosphorylation of Serine 268 (Ser268) residue by an extracellular signal-regulated kinase 1 and 2 (ERK1/2), leads to a change in the size of gephyrin scaffold at postsynaptic site (Tyagarajan et al., 2013). In parallel, these changes, seen with immunochemistry techniques, influence also the amplitude and frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSC). These are only two examples, by which post-translational modification of gephyrin can fine-tune $GABA_A$R-mediated synaptic-transmission.

The identification of Ser268 residue on gephyrin as a target site for acetylation, in addition to act as phosphorylation site, suggest that this could be another regulatory mechanism, in determining the phosphorylation status of gephyrin and therefore controlling GABAergic transmission (Tyagarajan et al., 2013).

Although several possible acetylation residues on gephyrin were identified by mass-spectrometry, little is presently known about the impact on gephyrin acting as a scaffolding protein at GABAergic synapses (Schwer et al., 2009, Tyagarajan et al., 2013).

Further, our group has shown that sumoylation of gephyrin has an effect on gephyrin clustering at postsynaptic sites.

Altogether, these findings demonstrate that post-translation modification mechanism, influencing the clustering of gephyrin at the postsynapse, is important for efficient GABAergic neurotransmission by regulating the density of $GABA_A$Rs at the synapse.

1.6 Gephyrin and postsynaptic GABAergic synaptic plasticity

As mentioned above, gephyrin clustering at GABAergic synapses is a dynamic process and critical for proper GABAergic neurotransmission. Extracellular signals due to a change in network activity, like Ca^{2+}-influx following moderate stimulation of neurons, is enough to activate ERK1/2 leading to a decreased size in gephyrin clusters and mIPSC amplitudes (Marsden et al., 2007, Marsden et al., 2010, Tyagarajan et al., 2013). Similarly, Ca^{2+} can activate the protease calpain which promotes the degradation of gephyrin clusters and therefore weaken GABAergic synapse (Tyagarajan et al., 2011b). The fact that gephyrin interacts with many proteins, such as CB, NL2 but as well with profilin, an actin-binding protein which is involved in the dynamic turnover and restructuring of the actin cytoskeleton, suggest that changes in the gephyrin scaffold might affect down-stream signaling contributing to structural changes and GABAergic synaptic plasticity. However, there is only limited evidence for a direct interaction of gephyrin and profilin isoforms resulting in dynamic modifications of the actin cytoskeleton (Mammoto et al., 1998, Murk et al., 2012). It is more likely that the binding of gephyrin to CB, leads to a down-stream signaling via Cdc42 which results in a change in the actin dynamics (Grosskreutz et al., 2005). However, a recent article demonstrated a correlation between gephyrin phosphorylation, and therefore its clustering properties, and the rearrangement of the cytoskeleton after changes in neuronal activity (Rui et al., 2013). In cultured hippocampal neurons, they identified GSK3β, which targets gephyrin residue Ser270, as a key player in GABAergic activity-dependent regulation of dendritic growth and stabilization. This data

demonstrates a signaling cascade, possibly triggered by a disrupted E/I-balance, that the control of the phosphorylation status of gephyrin regulates not only the aggregation of $GABA_ARs$ at the plasma membrane but also the interaction with other signaling molecules within and/or surrounding the PSD, leading to a reorganization of the cytoskeleton.

These observations together make it clear that there are several mechanisms up- and down-stream of gephyrin leading to GABAergic synaptic plasticity and that both the conformation and concentration of PSD proteins, as well a proper functional interaction between these molecules within the PSD is crucial.

2 Adult neurogenesis

Neurogenesis refers to the process of generating new neurons, arising from neural stem and progenitor cells (NSC and NPC). For more than half of the last century it was considered that neurogenesis occurs only during embryonic development and perinatal stages in the mammalian CNS (Ming and Song, 2005). In 1962, Altman and Das reported for the first time, using ^3H-thymidine autoradiography, the presence of adult-generated neurons in the rat hippocampus and neocortex. This observation was largely ignored by the scientific community, however, with the discovery of bromodeoxyuridine (BrdU), several studies revealed the continuous production and functional integration of new neurons in the mature nervous system. Nowadays, adult neurogenesis is widely accepted and has been described in rodents, fishes, lizards, insects, birds, reptiles, monkeys and also in humans (Luskin, 1993, Lois and Alvarez-Buylla, 1994, Eriksson et al., 1998, Kempermann and Gage, 1999, Gage, 2000, Gross, 2000, Picard-Riera et al., 2002, Alvarez-Buylla and Lim, 2004, Ming and Song, 2005, Bergmann et al., 2012, Spalding et al., 2013). In the adult mammalian CNS, adult neurogenesis mainly occurs in the dentate gyrus (DG) of the hippocampus and in the OB, and additionally in the olfactory epithelium. It is a highly-coordinated process, including several distinct steps starting with stem cell maintenance, proliferation of NCSs and NPCs, neuroblast fate specification and migration, differentiation, survival and integration into the existing neuronal network. All these steps are under precise spatial and temporal control, but can be influenced by both internal and external stimuli, which either act directly onto the cells or indirectly by modulating existing network activity (Petreanu and Alvarez-Buylla, 2002, Kempermann et al., 2004, Ming and Song, 2005, Yamaguchi and Mori, 2005, Zhao et al., 2008, Ma et al., 2009, Mouret et al., 2009). Several studies have identified a number of molecular players and signaling pathways which contribute to the regulation of different aspects of adult neurogenesis, including transcription factors (paired box 6

(PAX6), Krüppel-like factor 9), epigenetic factors, trophic factors (brain-derived factor (BDNF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF)), hormones, adhesion molecules (neural cell adhesion molecule (NCAM), Slit), cytokines, neurotransmitter (GABA, glutamate, serotonin), Notch signaling. Moreover, environmental factors such as age, antidepressants, odors, chronic stress, injuries, physical activities and learning, can influence adult neurogenesis (reviewed by (Ming and Song, 2005, Lledo et al., 2006, Zhao et al., 2008, Sun et al., 2011)). The ability of the brain to adapt to internal and external environmental changes by adding adult-born neurons in order to fine tune existing circuitries, revealed a huge capacity of plasticity.

Today, it is well known that GABAergic signaling is important in regulating brain development early in life, but also that GABA exerts a fundamental role in regulating distinct steps of adult neurogenesis.
In the next chapter, I will illustrate the importance of GABAergic transmission trough $GABA_A$Rs in modulating and controlling proliferation of NSCs and NPCs, migration, neuronal differentiation and integration in the OB as well as DG of the hippocampus. Therefore, I briefly present the cytoarchitecture of the OB and the hippocampal formation with a focus on their neuronal and synaptic organization.

2.1 Olfactory bulb

The OB is the first center in the CNS which transmits odor information coming from the nose to higher brain areas. The OB has a characteristic multi-layered cellular organization; from outer to the center, 5 layers can be distinguished: glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL) and granule cell layer (GCL).
After an air-spread molecule is bound to a specific odorant receptor expressed by olfactory sensory neurons (OSN) in the olfactory epithelium, an electrical signal transduces the chemical information via the axons of the OSNs towards the GL. The GL represents a topographical odor map, organized in single glomeruli (Ressler et al., 1994, Mombaerts et al., 1996, Mori et al., 1999). Each OSN expresses only one out of thousand olfactory receptors and sends its axon into a single glomerulus, along with all sensory neurons expressing this olfactory receptor (Buck and Axel, 1991). Within the glomeruli, OSNs make excitatory glutamatergic synapses with the apical dendrite of mitral cells and tufted cells (M/T), the main projection neurons of the OB. Before the odor information reaches higher brain centers through the axons of M/T cells, the input

information further get processed by local circuit neurons (PGCs, GCs). PGCs are mainly GABAergic and to a smaller extend dopaminergic axonless neurons, which form reciprocal dendro-dendritic synapses with M/T cells. Their dendrites terminate either in a single glomerulus or in several glomeruli, enabling them to modulate signaling between different glomeruli. Further, in the EPL, GCs, characteristically axonless and the main type of bulbar GABAergic interneurons, control the output of M/T cells by forming as well reciprocal dendro-dendritic synapses. These bidirectional synapses include $GABA_AR$ containing $\alpha1/\alpha3$ subunits (Panzanelli et al., 2005) in the MC dendrite and ionotropic glutamate receptors (AMPA and NMDA) in the GC spine. This synaptic formation leads to strong lateral inhibition with M/T cells, which is important for M/T cell synchronization and odor discrimination (Price and Powell, 1970, Yokoi et al., 1995, Shepherd et al., 2007). Furthermore, GCs receive glutamatergic inputs form M/T cells and centrifugal fibers coming from anterior olfactory nucleus, piriform, periamygdaloid and lateral entorhinal cortex, and GABAergic inputs from short-axon cells controlling the excitability of the GCs, mediated by the $\alpha2$- and $\alpha3$-$GABA_AR$ subunit (Fritschy and Mohler, 1995, Panzanelli et al., 2009). Finally, axons of M/T cells transmitting the odor information, now processed and modulated through bulbar microcircuits, form a bundle- the lateral olfactory tract- that targets first the olfactory cortex, anterior olfactory nucleus, piriform and lateral entorhinal cortex and amygdala and in addition secondary olfactory areas including hippocampus, hypothalamus, thalamus and cerebellum (Scott et al., 1980, Zald and Pardo, 2000, Ma, 2007, Ghosh et al., 2011, Miyamichi et al., 2011).

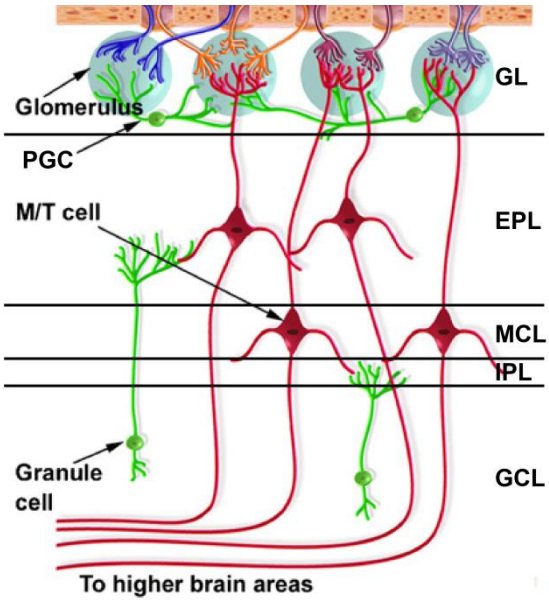

Figure 3 Schematic representation of the OB cytoarchitecture
ONS (blue, orange, purple, violet) project to the GL where they make synapses on OB projection neurons (MC and TC) within specific glomeruli. Interneurons, such as PGCs in the GL and GCs in the GCs modulate the projection neurons before their axon send the information toward higher cortical centers. GCs are also regulated by local interneurons (short axon cells) (modified from Adam and Mizrahi, 2009).

2.2 Hippocampus

The hippocampal formation is part of the medial temporal lobe of the brain and plays a crucial role in the formation of explicit long-term memory, short-term declarative memory, learning and spatial navigation (O'Keefe and Dostrovsky, 1971, Squire, 1992, Kesner, 2007). Through its interconnections with different brain areas, the hippocampus plays also a role in emotional processing and odor discrimination (Eichenbaum et al., 1989). The hippocampal formation consists of 3 substructures with its distinct principal cells: the DG including GCs, the hippocampus proper (CA1, CA2, and CA3 fields) containing pyramidal neurons, and the subiculum. The synaptic connections between these structures build the excitatory trisynaptic pathway of the hippocampal neural network. Information from cortical areas bypassing the layer II of the entorhinal cortex flows through the perforant pathway to the GCs in the DG. The axons of the GCs project to the pyramidal cells in the CA3 via the mossy fibers. From there on, the information relays through the Schaffer collaterals to the pyramidal cells in CA1 which project then back to the subiculum and entorhinal cortex (Deng et al., 2010, Benarroch, 2013). However, direct synaptic inputs from the entorhinal cortex to the CA1 and CA3, as well as from various brain regions to the DG (dorsal raphe nucleus, septal nucleus, and ventral tegmental area), make the hippocampal network highly dynamic (Song et al., 2012a). Another major cell population, which modulates the excitability of the trisynaptic circuit, are GABAergic inhibitory interneurons generating field potential oscillations. Beside the different synaptic connections which modulate the adult hippocampus circuitry, the continuous addition of adult-born neurons has the ability to modify the local neural network (Song et al., 2012a). The functional contributions of this dynamic network leading to memory acquisition and retention of hippocampal formation are not yet fully understood.

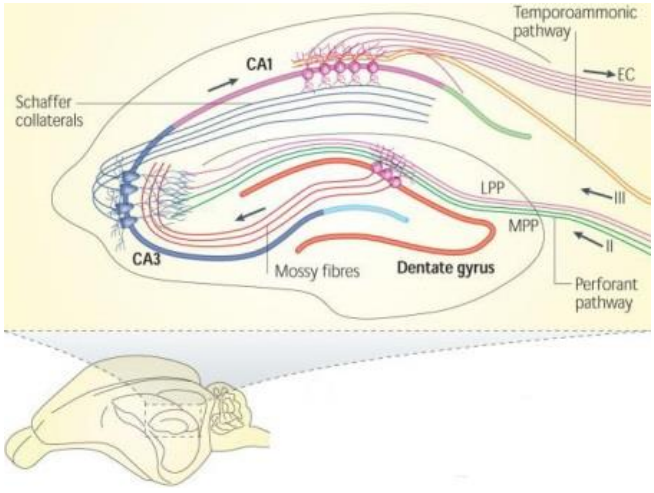

Figure 4 Diagram of hippocampal trisynaptic pathway
Excitatory afferent fibers form the EC project via the lateral and medial prefrontal pathway (LPP and MPP) to the DG. DG-GCs send projections via the mossy fiber tract toward the pyramidal cells in the CA3. Through the Schaffer collaterals signals are further transmitted to the CA1 pyramidal cells and then back to the EC (Deng et al., 2010).

2.3 Role of GABAergic transmission in regulating distinct steps of adult neurogenesis

Throughout life, new neurons are generated and synaptically-integrated in the DG of the hippocampus and in the OB of all mammals. In both systems, there are common and different mechanisms regulating the process from birth of adult-born neurons to their functional integration into existing networks. It is known that GABAergic neurotransmission regulates the entire process, from proliferation and migration to differentiation and synaptic integration, in both systems. In the following paragraph, I will highlight the different steps of adult neurogenesis, with a focus on the role of GABAergic signaling.

Proliferation and fate specification

In the adult brain, NPCs are localized within specialized neurogenic niches, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the DG (Gage, 2000, Alvarez-Buylla et al., 2001, Rakic, 2002). Adult NPCs exhibit astrocytic characteristics and have the capability to self-renew, be quiescent or differentiate either into astrocytes, oligodendrocytes or neurons (Doetsch et al., 1999, Seri et al., 2001, Bonaguidi et al., 2011).

Upon activation of quiscent radial glia-like NSCs, named type B-cells in SVZ and type-1 cells in the SGZ, they differentiate into type C transient amplifying cells and intermediated progenitor cells (type-2; SGZ). Further, these cells give rise to migrating neuroblasts; called type A in the SVZ (Abrous et al., 2005). In the SVZ and in the SGZ, precursor cells and neuroblasts are already sensitive to GABA, however in a non-synaptic manner. In the SVZ, they release ambient GABA, which leads to the auto-control of NPC proliferation through the activation $GABA_A$Rs on their cells surface (Stewart et al., 2002, Wang et al., 2003, Bolteus and Bordey, 2004, Liu et al., 2005). Quiescent NPCs in the SGZ respond as well, through $GABA_A$Rs (containing the γ2-subunit presumably associated with α5-and β3-subunits) tonically to ambient GABA, whereas here GABA is released by parvalbumin (PV)-positive interneurons. It has been demonstrated that γ2-subunit conditional knockout (KO) mice exhibit an increase in cell proliferation and renewal of stem cells (Song et al., 2012b). In addition, α4-KO mice showed an increase in NPC proliferation (Duveau et al., 2011). These observations suggest that in both systems, $GABA_A$R activation through tonic currents is a negative feedback mechanism regulating proliferation rate and maintaining the NSC pool.

Migration and differentiation

Neuroblasts from the SVZ migrate tangentially, by forming a chain consisting of a group of 30-40 cells, over a long distance in the rostral migratory stream (RMS) toward the OB, where they differentiate mainly as GABAergic GCs and to a smaller extent PGCs (Lledo et al., 2006). In contrast, neuroblasts from the SGZ migrate only for a short distance (a few microns) into the inner GCL of the DG before differentiating as excitatory GCs (Lois et al., 1996).

Ambient GABA, released by OB neuroblasts, reduces the speed of migration, shown in acute mouse slices where GABA release was enhanced (Bolteus and Bordey, 2004). Moreover, NKCC1 activity, which is responsible for the depolarizing action of GABA, is necessary for maintaining normal migratory speed (Mejia-Gervacio et al., 2011).

Whereas GABA signaling controls the speed of migration in the RMS, GABA plays a crucial role in the final positioning of DG-GCs in the GCL. Our lab has shown that the lack of the α4-$GABA_AR$, and therefore partial tonic currents, in α4-KO mice, curtails the migration distance compared to wild-type mice. Conversely, neuroblasts in α2-KO mice migrate deeper into the GCL (Duveau et al., 2011).

Maturation and synaptic integration of adult-born GCs

A common method to analyze OB-GC development is the injection of an eGFP lentivirus into the RMS to birth-date adult-born GCs. In contrast, to monitor DG-GC development, typically retroviruses are injected into the SGZ, to infect dividing cells (Papale et al., 2009). The full integration of new neurons into the existing circuitry is terminated within a few weeks and comes along with structural plasticity. However, a massive number of adult-born neurons do not become integrated (~50% in the OB; ~60-80% in the DG) but instead undergo programmed cell death (Cameron and McKay, 2001, Petreanu and Alvarez-Buylla, 2002). Likewise here, GABAergic signaling is critical for the proper maturation and integration of both OB- and DG-GCs.

After adult-born neurons reach the OB (1-2 days post-virus injection (dpi)), they detach from the RMS and migrate radially in the GCL to their final position. Upon entering in the GCL, GCs extend a single apical dendrite toward the MCL, and rapidly receive axo-dendritic synapses, with a predominance of GABAergic versus glutamatergic transmission (Carleton et al., 2003, Panzanelli et al., 2009). The GABAergic inputs arrive from local interneurons (short-axon cells) and are mediated mainly through the α2-containing $GABA_ARs$, which are clustered at postsynaptic sites of GCs, along with gephyrin. Glutamatergic inputs coming from centrifugal

fibers and M/T cell axons are mainly found on spines of GCs. However, it has been proposed that GABAergic synapses onto immature GCs are formed transiently on spines, whereas in mature cells, the majority of GABAergic contacts are present on the shaft (Panzanelli et al., 2009). With onward maturation, the apical spiny dendrite crosses the MCL and starts branching within the EPL, accompanied with the formation of dendro-dendritic reciprocal synapses. At this stage glutamatergic synaptic transmission begins to dominate. Whereas, synaptogenesis in adult-born OB-GCs occurs very fast, the consequences on the morphological maturation is much slower. It has been reported that OB-GCs reach a maximal number in dendritic branching, spines and synapses, after 30 dpi but then it declines between 30 and 56 dpi (Whitman and Greer, 2007, Livneh and Mizrahi, 2012, Pallotto et al., 2012). This dynamic in plasticity is reported as characteristic of maturing adult-born GC, because in mature GCs, at 90 dpi, these striking changes are not detectable anymore (Nissant et al., 2009).

Due to the fact that shortly after entering into the OB, adult-born GCs respond mainly through the α2-$GABA_AR$ to phasic GABAergic transmission, suggest that this receptor is important in controlling maturation. Indeed, Pallotto et al. showed, by targeted deletion of the α2 subunit in OB-GCs, that a strong reduction in spontaneous and evoked inhibitory postsynaptic currents (eIPSCs) negatively affects GCs development.

In the DG, adult-born GCs send their dendrites into the molecular layer, where they first receive slow GABAergic inputs from interneurons (Song et al., 2012a). At this time GABA has a depolarizing action, due to the NKCC1 expression on immature DG-GCs. Later (~ 21 dpi), they get glutamatergic inputs from the entorhinal cortex via the perforant pathway. Further, the DG-GCs send their axons along the mossy fiber pathway to the CA3 where they target principal cells (Jessberger et al., 2008b, Zhao et al., 2010). DG-GCs undergo, as well as OB-GCs, a change in structural reorganization during maturation. Until ~ 21 dpi, DG-GCs are spineless but then start with a sharp increase in spine density between 21-28 dpi. Between 28-42 dpi, GCs undergo a critical period where the depolarizing action of GABA enables the activation of NMDA receptors, in the absence of AMPA receptors. With the gradual maturation and the increased synaptic connectivity, adult-born DG-GCs have a potential for synaptic plasticity, including long-term potentiation (LTP). At later stages (42 dpi), with the loss of prominent structural plasticity and by the transition of GABA from excitatory to inhibitory, neurons can be considered to be mature (Ge et al., 2006, Zhao et al., 2006, Toni and Sultan, 2011).

Duveau et al. have demonstrated that the GABAergic signaling via the α2- and α4-$GABA_A$Rs are critical in the early and late morphological development of DG-GCs. At 14 dpi adult-born neurons of α4-KO mice show a prominent reduction in dendritic ramification, whereas in α2-KO mice the initial dendritic growth was normal. However, at 42 dpi, the absence of the α2 subunit causes also a significantly reduced dendritic complexity, which can be prevented by pharmacologically reducing the number of glutamatergic inputs onto these cells. In conclusion, different $GABA_A$R subtypes are able to fulfill specific stages of adult-born GC development in both neurogenic regions.

2.4 Functional role of adult neurogenesis

During the past 20 years, various intrinsic and extrinsic signals have been identified in regulating adult neurogenesis. Nevertheless, the functional impact of this process on the behavior and physiology remains largely elusive. Several studies showed that stress, social isolation, physical activity and environmental enrichment can stimulate or impair adult neurogenesis (van Praag et al., 2000, Ming and Song, 2011, Wolf et al., 2011, Yau et al., 2011). A variety of different experimental protocols have been used to alter the levels of neurogenesis in both systems, as a consequence with unequal interpretations. However, all experimental evidence indicates that the adding of adult-generated neurons in the DG and OB is relevant for learning and memory (Deng et al., 2010). In particular, activation of mouse adult-born OB-GCs expressing channelrhodopsin, led to improved odor discrimination learning and memory (Alonso et al., 2012). In contrast, disruption of olfactory adult neurogenesis in female mice resulted in abnormal social interaction with male conspecifics suggesting an inability to detect or discriminate male-specific odors (Feierstein, 2012).

Recent experimental evidence has indicated that adult neurogenesis in the DG plays a fundamental role in pattern separation (Tronel et al., 2012). Mice with an increased production of adult-born neurons in the DG, show normal object recognition, but performed better in distinguishing between similar contextual representations in a behavioral paradigm (Sahay et al., 2011). Interestingly in both systems, environmental enrichment causes an increase in neurogenesis, whereas environmental deprivation or social isolation reduces the number of newborn neurons (Lieberwirth et al., 2012, Lepousez and Lledo, 2013).

Moreover, nowadays it is well known that NPC proliferation, neuronal differentiation, and survival of newborn neurons are dramatically affected with age.

Furthermore, under pathological conditions, alterations in distinct processes of adult neurogenesis are seen, especially in neurodegenerative diseases, like Alzheimer disease, Parkinson disease, or epilepsy (Mirochnic et al., 2009, Winner et al., 2011, Gallarda and Lledo, 2012). This implicates that mechanisms of neurodegenerative diseases are coupled to brain plasticity. Epilepsy-induced seizures contribute to an increased proliferation of adult-born neurons, however most of those GCs failed to migrate, differentiate and integrate properly (Kralic et al., 2005, Ledergerber et al., 2006, Scharfman and Hen, 2007). In addition, in animal models of depression, hippocampal neurogenesis obtained attention, especially the role of antidepressant drugs.

Further, alterations of GABAergic transmission, inducing abnormal circuit formation, are most likely related to pathophysiology of various brain diseases. Therefore, it is of major interest to investigate the role of signaling cascades regulating GABAergic transmission to understand the mechanisms which might contribute to neurological diseases. In particular, several NL and neurexin genes have been implicated to play a role in the development of schizophrenia and autism spectrum disorders (Sudhof, 2008, Bang and Owczarek, 2013). Additionally, it was suggested that CB is involved in the development of X-linked mental retardation associated with epilepsy and anxiety (Kalscheuer et al., 2009). Therefore, studying GABAergic synapses in a model system of adult neurogenesis is a way to understand the complex interaction of synaptic circuits and its consequence on neuropathological diseases. Beyond that, understanding the different processes of adult neurogenesis might be clinically relevant for new therapeutic interventions and possibly for neuronal cell translation therapy, especially NSCs.

II. AIM OF THE THESIS

GABAergic synapses exhibit dynamic properties that enable strengthening or weakening GABAergic transmission upon alterations in network activity and changes in E/I-balance. Multiple signaling pathways converge onto gephyrin, the main scaffolding protein of GABAergic synapses, to adjust its clustering properties by post-translational modification, and therefore its interactions with $GABA_A$Rs. Further, gephyrin interacts with down-stream signaling molecules, which in turn can induce functional and structural adaptations at the synapse. While these mechanisms have been fairly well characterized *in vitro*, using primary neuronal cultures, their relevance *in vivo* remains largely speculative.

In addition to its role as main inhibitory neurotransmitter in adult CNS, GABAergic transmission plays an important role during ontogeny. These effects are recapitulated during adult neurogenesis, and $GABA_A$R-mediated transmission has been shown in adult animals to control neural stem and precursor cell proliferation, neuronal migration, dendritic maturation, and synaptic integration into pre-existing circuits. Therefore, adult neurogenesis provides a powerful system to test the relevance of GABAergic synaptic plasticity, orchestrated by gephyrin and $GABA_A$Rs, for CNS development and function.

In my PhD thesis, I have used two distinct approaches to investigate the significance of GABAergic synapse plasticity for the regulation of adult neurogenesis. In the first study, I investigated the effects of gephyrin mutations, introduced selectively in adult-born neurons by viral transfection, on dendritic maturation, synapse formation, and synaptic plasticity of adult-born OB-GCs. This work allowed to provide direct evidence that the clustering properties of gephyrin are decisive for activation of signals required for proper neuronal maturation. In the second study, I investigated the effects of targeted gene deletions affecting a specific $GABA_A$R subtype mainly mediating tonic inhibition on the migration and morphological maturation of adult-born GCs of the DG. These results allowed, in particular to evaluate the importance of down-stream signals activated by extra-synaptic $GABA_A$Rs. By comparing the alterations in neuronal development observed in both sets of experiments, this study will help unraveling the significance of the dynamic regulation of GABAergic synapses for proper CNS maturation and function.

Study I **Postsynaptic gephyrin clustering controls the development of adult-born granule cells in the olfactory bulb**

A previous study reported that targeted deletion of the α2-$GABA_A$R in adult-born OB neurons negatively affects their morphological development, as well as their synaptic integration. Even sensory enrichment could not rescue the phenotype indicating that GABAergic transmission mediated through α2-$GABA_A$Rs are required for proper GC maturation. However, since the deletion of the α2 subunit also caused a disruption of postsynaptic gephyrin clusters, some of the effects observed could be related to the loss of signaling molecules at the GABAergic PSD. Therefore, we aimed here to test the role of gephyrin clustering in regulating adult-born GC differentiation. To this end, we used lentivirus-mediated over-expression of gephyrin and gephyrin mutant constructs in adult-born GCs and monitored their differentiation and synaptic integration by morphological analysis.

Study II **A partial, but not a full, inactivation of α5 subunit-containing $GABA_A$ receptors in mice affects the development of adult-born neurons in the dentate gyrus**

Tonic inhibition mediated by extra-synaptic $GABA_A$Rs is an important regulator of hippocampal adult neurogenesis, as well as a key determinant of learning and memory. In particular, negative allosteric modulation of α5-$GABA_A$Rs can improve cognitive performance in models of intellectual disability. A possible role of these receptors in the control of adult neurogenesis has not been determined, although there is evidence that α4-$GABA_A$Rs control proliferation, migration, and dendritic development of adult-born DG-GCs. The aim of this study was to determine the effects of global and partial loss of α5-$GABA_A$Rs, using α5-KO, α5-het mice, respectively, on the development of adult-born GCs and to test if these effects are cell-autonomous by inducing a cell-specific deletion of the *Gabra5* gene using the Cre-lox system. To this end, we used retroviruses expressing eGFP to birth-date adult-born GCs in the DG and monitor their morphological differentiation at specific stages of maturation.

III. RESULTS

STUDY I: POSTSYNAPTIC GEPHYRIN CLUSTERING CONTROLS THE DEVELOPMENT OF ADULT-BORN GRANULE CELLS IN THE OLFACTORY BULB

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Running Head: Gephyrin regulation of neuronal development
Key Words: Adult neurogenesis; dendrites; GABA; postsynaptic density; phosphorylation; spines.

I performed all experiments, some with help of MP, FV, MG, MAV, and PP, analyzed data and wrote the paper with help of SKT and JMF.

Abstract

In adult olfactory bulb, GABAergic signaling regulates migration, differentiation, and synaptic integration of newborn granule cells (GCs), migrating from the subventricular zone. Targeted deletion of the $GABA_A$-receptor ($GABA_AR$) α2-subunit in these cells abolishes fast synaptic GABAergic transmission and disrupts the postsynaptic scaffold organized by gephyrin. Consequently, mutant GCs exhibit major deficits in morphological differentiation and synapse formation. Here, we investigated possible contributions of the gephyrin scaffold, which anchors $GABA_ARs$ and various classes of signaling molecules at GABAergic postsynaptic densities, to the differentiation and long-term survival of adult-born GCs. This goal was achieved by over-expression of gephyrin mutant constructs tagged with eGFP. Lentiviral vectors encoding either eGFP-gephyrin (eGFP-Geph), or phospho-deficient eGFP-Geph(S270A) (which favors the formation of supernumerary GABAergic synapses *in vitro*), or dominant-negative eGFP-Geph(L2B) (a mutation that prevents gephyrin from clustering) were injected into the rostral migratory stream of adult C57BL6/J mice to target migrating neural progenitor cells. Over-expression of eGFP-Geph, as examined between 2 and 12 weeks later, had almost no detectable effects as compared to eGFP alone, whereas eGFP-Geph(S270A) favored dendritic branching and formation of transient GABAergic synapses on spines, containing α2-$GABA_ARs$. Strikingly, over-expression of eGFP-Geph(L2B) had the opposite effect and strongly impaired overall morphological development. Such GCs had shorter survival, due to increased early and late mortality rates. These results unravel a role for gephyrin phosphorylation and postsynaptic clustering in regulating GABAergic synapse formation and spinogenesis. Therefore, signaling at GABAergic synapses, orchestrated by the gephyrin scaffold, contributes to morphological differentiation and long-term survival of adult-born olfactory bulb neurons.

Introduction

Adult neurogenesis is an important facet of brain plasticity and offers a window for investigating the molecular and cellular mechanisms regulating the differentiation of newborn neurons and their functional integration into existing circuits (Whitman and Greer, 2009, Ming and Song, 2011, Lepousez et al., 2013, Pallotto and Deprez, 2014). In the olfactory bulb (OB), adult neurogenesis sustains the constant replacement of granule cells (GCs) and periglomerular cells, which represent two distinct populations of interneurons regulating the activity and synchronization of principal cells (mitral and tufted cells) (Lledo et al., 2006, Lagier et al., 2007). GCs are axonless GABAergic interneurons located in the granule cell layer (GCL), which have a spiny apical dendrite establishing reciprocal, dendro-dendritic synapses with mitral and tufted cells in the external plexiform layer (EPL) (Shepherd et al., 2007). GCs receive glutamatergic and GABAergic inputs from various sources, mainly distributed on spines and dendritic shafts, respectively. There is considerable evidence that their differentiation and synaptic integration (afferent and efferent) in adult brain is regulated by GABAergic signaling (reviewed in (Alonso et al., 2006, Gascon et al., 2006, Bonin et al., 2007, Platel et al., 2007, Bonaguidi et al., 2011, Nissant and Pallotto, 2011)), but the underlying mechanisms are not clear.

We have shown in a previous study that targeted deletion of *Gabra2* in adult-born GCs, preventing the formation of a major $GABA_A$ receptor ($GABA_AR$) subtype expressed by these neurons, curtails their morphological differentiation and strongly reduces the formation of afferent and reciprocal synapses on spines (Pallotto et al., 2012). Further, these mutant GCs are also compromised in their ability to remodel dendrites and synapses in response to sensory enrichment or deprivation, suggesting a key role for α2-$GABA_ARs$ in regulating neuronal development and plasticity. Importantly, α2-$GABA_ARs$ appear to be dispensable for regulating migration of precursor cells in the rostral migratory stream (RMS) and initial dendrite and synapse formation (up to about 2-3 weeks post final mitotic division) (Pallotto et al., 2012).

Targeted deletion of *Gabra2*, besides affecting the formation of α2-$GABA_ARs$, also prevented postsynaptic clustering of gephyrin, the major scaffolding protein of GABAergic and glycinergic postsynaptic densities. Gephyrin is known to interact with a number of signaling molecules, notably protein kinases and phosphatases, proteases such as calpain, the RhoGEF collybistin, and modulators of the actin cytoskeleton (reviewed in (Tyagarajan and Fritschy, 2014)). In turn, its postsynaptic clustering is regulated by post-translational modification, in particular

phosphorylation of specific, identified residues. Among these, several lines of evidence indicate that gephyrin phosphorylation on Ser270 modulates GABAergic synaptic function and dendritic development (Panzanelli et al., 2011, Kuhse et al., 2012, Rui et al., 2013). Therefore, we reasoned here that some of the impairments of adult-born GC maturation observed in the absence of α2-$GABA_A$Rs might be secondary to the disruption of the postsynaptic density organized by gephyrin, leading to dispersion (and loss of function) of signaling molecules that interact with the gephyrin scaffold.

To test this hypothesis, and more generally to investigate the role of gephyrin in regulating neuronal differentiation and plasticity, we used here lentivirus- (LV)-mediated over-expression of gephyrin and gephyrin mutants, N-terminally fused to eGFP, in adult-born olfactory GCs. The gephyrin constructs tested in this study include wild-type rat gephyrin (P2; Geph), Geph(S270A), which contains a point-mutation abolishing phosphorylation of residue Ser270 (Panzanelli et al., 2011), and Geph(L2B), which is engineered with a 6 amino acid substitution in a surface-exposed loop (L2B) of the gephyrin E-domain, known to abolish gephyrin clustering in neurons and to exert a dominant-negative effect on endogenous gephyrin clustering (Lardi-Studler et al., 2007).

LVs encoding these different gephyrin isoforms were injected into the RMS of adult wild-type mice to selectively transduce adult-born GCs. In combination with immunohistochemical staining for light- and electron-microscopy, we investigated the impact of gephyrin over-expression and clustering on the maturation and synaptic integration of adult-born GCs, as well as on their long-term survival. Specifically, we focused on dendrite development, spine growth, and GABAergic synapse formation, expecting eGFP-Geph(S270A) to facilitate these processes and eGFP-Geph(L2B) to impair them.

Our data indicate that adequate gephyrin clustering is necessary for proper neuronal development and survival, emphasizing the crucial role played by the gephyrin scaffold for organizing signaling cascades down-stream of GABAergic synapses.

Materials and Methods

Animals

All experiments were performed in accordance with the European Community Council Directives of November 24, 1986 (86/609/EEC) and approved by the cantonal veterinary office of Zurich. In the present study, we used 8 to 12 week-old male C57BL6/J mice, which were bred in the animal facility of the Institute of Pharmacology and Toxicology at the University of Zurich.

Lentiviral vectors

LVs encoding the eGFP constructs were produced by transfecting HEK 293T cells with four separate plasmids containing the transgene under the control of the phosphoglycerate kinase (PGK) promoter (eGFP, eGFP-Geph, eGFP-Geph(S270A) and eGFP-Geph(L2B)), packaging and coat. The supernatant containing the virus was concentrated by ultracentrifugation and diluted in PBS. Typically, the preparations used had a titer of 10^8 cfu/mL, as determined upon HEK293 cell transfection. The LV encoding tdTomato (UbC-TdTomato-WPRE; origin plasmid: FUtdTW (Addgene Plasmid #22478) was generously provided by the lab of Dr. P.M. Lledo (Institut Pasteur, Paris). Its titer, determined by qPCR, was $8*10^9$ VG/mL.

Stereotaxic injections

Adult male mice were anesthetized by inhalation with 2.5-3% isoflurane (Baxter) in oxygen and placed on the stereotaxic frame (David Kopf Instruments). The mice received a bilateral injection of viral particles (200 nL) into the RMS (anteroposterior (AP) = +3.3 mm, mediolateral (ML) = ±0.82 mm, dorsoventral (DV) = -2.9 mm, with Bregma as reference), using a nanoliter injector Nanoject II (Drummond Scientific). After the surgery, the animals received an i.p. injection of 1 mg/kg buprenorphine (Temgesic, Essex Chemicals, Lucerne, Switzerland) and were recovered from anesthesia on a warm pad, before returned to their home cage.

Tissue preparation for immunohistochemistry

Mice were deeply anesthetized with pentobarbital (Nembutal®; 50 mg/kg, i.p.) and perfused transcardially with 15-20 mL ice-cold, oxygenated artificial cerebrospinal fluid (ACSF), pH 7.4, as described (Notter et al., 2014). After perfusion, the mice were decapitated immediately on ice and the OB was dissected out and fixed by immersion in 4% paraformaldehyde (dissolved in 0.15 M sodium phosphate buffer, pH 7.4) for 90 min. After cryoprotection overnight in 30%

sucrose, 50-µm-thick horizontal or coronal sections from frozen OB were cut with a sliding microtome and collected in phosphate-buffered saline (PBS).

Immunofluorescence staining

Double and triple immunofluorescence staining was performed by incubating OB sections with the primary antibody (Table 1) diluted in PBS (pH 7.4) containing 2% normal donkey serum and 0.2% Triton X-100 for 48-72 h at 4°C.

Sections were then washed in 3x PBS for 10 min and incubated at room temperature in secondary antibodies. Secondary antibodies conjugated to Alexa 488 (Invitrogen), Cy3, or Cy5 (Jackson ImmunoResearch) were raised in donkey. Afterwards, sections were rinsed again 3 times in PBS and mounted on gelatin-coated slides, before coverslipped with a fluorescence mounting medium (Dako).

Table 1: List of primary antibodies

Target protein	Species	Dilution	Source; catalog	Method
GFP	ckicken	1:2000	Aves Laboratories; GFP-1020	IF
	rabbit	1:700	Synaptic Systems; 132 002	EM
Gephyrin	mouse	1:1000	Synaptic Systems; mAb7a; 147711	IF, EM
$GABA_AR$ α2-subunit	Guinea pig	1:1000	Self-made; Fritschy and Mohler, 1995	IF
vGAT	rabbit	1:3000	Synaptic Systems; 131 003	IF
	GP	1:10'000	Synaptic Systems; 131 004	EM
vGLUT1	rabbit	1:10'000	Synaptic Systems; 135 002	IF

Abbreviations: EM, immune-electron microscopy; IF, immunofluorescence

Lucifer Yellow experiment

Five mice injected with eGFP-Geph were deeply anesthetized with Nembutal® at 21 days post-injection (dpi) and perfused transcardially with ice-cold 0.9% NaCl followed by ice-cold 4% paraformaldehyde, 0.125% glutaraldehyde solution (pH 7.4). After postfixation for 2 h in 4% paraformaldehyde, 300-µm-thick horizontal OB sections were cut with a vibrating microtome and placed in an injection chamber in an Olympus microscope equipped with a

micromanipulator. Single eGFP-Geph-positive GCs visualized by epifluorescence were filled iontophoretically with a 0.4% Lucifer Yellow solution (Sigma-Aldrich, St. Louis, MO), using a sharp micropipette. A negative current of 70 nA was applied until the dendrites were fluorescing brightly.

To detect Lucifer Yellow, OB sections were incubated with rabbit antibodies to Lucifer Yellow (Invitrogen) diluted 1:4000 in PBS containing sucrose (5%), bovine serum albumin (2%), Triton X-100 (1%), and Na azide (0.1%), for 48 h at room temperature. Sections were then washed in PBS and incubated for another 48 h with a secondary antibody conjugated to Alexa 488 (Invitrogen; 1:1000). Afterwards, sections were washed in PBS, mounted and coverslipped with fluorescence mounting medium (Dako).

Image acquisition and analysis

Sections from all LV-injected mice were examined by epifluorescence microscopy after staining for GFP. Samples that contained either no eGFP-positive cells or cells distributed only in a small sector of the OB were discarded from further analysis. Data collection was done by laser-scanning microscopy (LSM700, Carl Zeiss GmbH, Germany), using sequential acquisition of separate wavelength channels to avoid fluorescence crosstalk.

To determine their survival rate, tdTomato-, eGFP-Geph(S270A)- and eGFP-Geph(L2B)-positive GCs were counted from 5-7 randomly selected coronal sections (anterior to the accessory olfactory bulb; 4-5 mice/group) and averaged per section. Dual acquisition mode was used to determine the fraction of double-labeled cells. The radial migration distance from the RMS was determined for a subset of 18-23 green and red cells/section, both as the absolute distance covered by the cell and as the relative fraction of the GCL thickness.

To investigate the total length and number of branches of the dendritic tree of adult-born GCs, GFP-positive GCs were selected randomly in the GCL (n=17-20 cells per mouse; 3-6 mice/group) from 50-um-thick sections. The entire cell was acquired, taking z-stacks spaced by 0.5 um. Dendritic morphometry was analyzed with the NeuronJ plug-in from the software NIH ImageJ.

Sholl analysis was performed with concentric circles, spaced at 10 μm intervals, centered on the cell body. The numbers of intersections were calculated using a Sholl Analysis plugin (Anirvan Ghosh Laboratory, University of California, San Diego, La Jolla, CA). For statistical comparison, the area under the curve (AUC) of the resulting function was calculated. Quantifications were performed on 13-20 cells from 3-6 mice of each experimental group.

The average number of spines, per unit length, on GFP-positive GCs (n=10-20 segments per mouse; 3-6 mice per construct) was calculated on 3D-projected image stacks with ImageJ software.

Localization of α2-subunit-positive clusters in spines or dendritic shafts of LV-infected GCs was determined manually in 3D images, using the co-localization module of the Imaris software (Bitplane, Switzerland; version 7.4). Quantification was done per unit length (measured on the same image). Total number of clusters was calculated based on average total dendritic length calculated in the morphometric analysis.

Preembedding electron microscopy

Stereotaxic injections of LVs were performed as described above in 6 mice per gephyrin construct assayed (eGFP-Geph, eGFP-Geph(S270A), eGFP-Geph(L2B)). At 21 dpi, they were deeply anesthetized with Nembutal and perfused with ice-cold 2% paraformaldehyde and 0.1% glutaraldehyde in sodium acetate buffer, pH 6, for 2 min, followed by 1 h perfusion with ice-cold 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M borate buffer, pH 9 (Kollo et al., 2008). After postfixation of the brain overnight in the second fixative, 70-µm-thick horizontal sections were cut with a vibrating microtome and stored in 0.1 M Na-phosphate buffer (pH 7.4). The sections were cryoprotected overnight in 30% sucrose and frozen/thawed three times with liquid nitrogen to enhance antibody penetration. Sections were then preincubated in 10% normal goat serum (NGS) in Tris-buffered saline (TBS), pH 7.4, for 3 hours at room temperature, followed by 5 days incubation at 4°C in primary antibodies (gephyrin/vGAT in combination with GFP) diluted in TBS, containing 5% NGS. Sections were then washed in TBS and incubated in secondary antibodies (Fab fragments) coupled to biotin (1:250; Jackson Immunoresearch) or to 1.4 nm colloidal gold particles (1:50; Nanoprobes Inc., Yaphank, NY) for 24 h. Ultrasmall gold particles were visualized with the gold enhance-EM formulation (Nanoprobes) as described by the manufacturer. The sections were treated with 0.5% OsO_4, and 1% uranyl acetate, dehydrated, and embedded in Epon 812. Ultrathin sections were collected on Nickel single-hole grids. Serial thin sections were cut in the EPL and GCL. eGFP-positive profiles were analysed in images taken from at least 6 sections at a magnification of 13,500x. They were observed and photographed in a JEM-1010 transmission electron microscope (Jeol, Japan) equipped with a side-mounted CCD camera (Mega View III, Soft Imaging System, Germany).

Statistical analyses

Data are presented as mean± SEM. Statistical analyses were made using one-way and two-way ANOVA, followed where appropriate by Tukey *post-hoc* tests (Prism software; GraphPad, version 6).

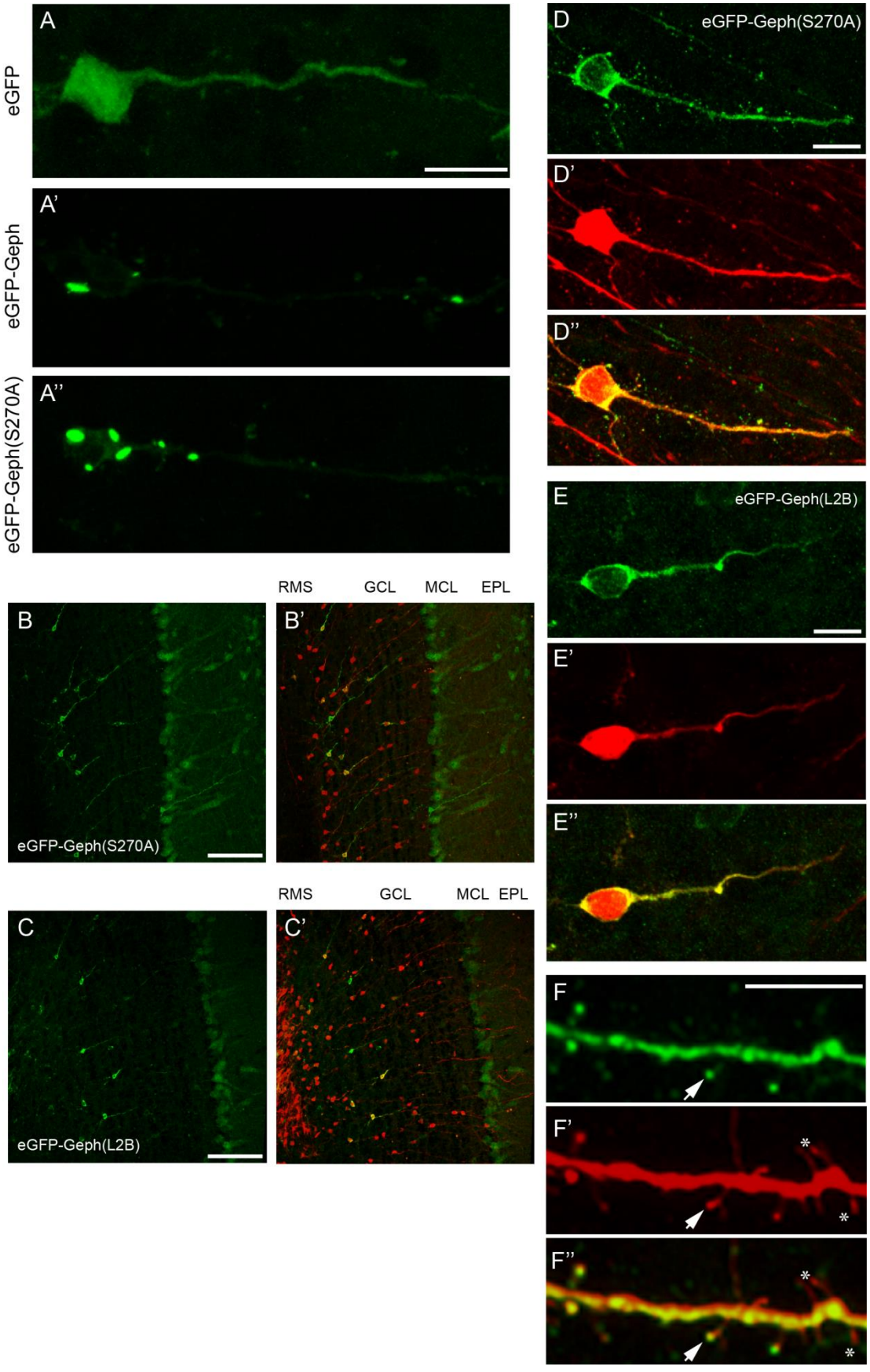
Results

Identification of adult-born GCs transduced by eGFP-gephyrin lentiviruses

The differentiation and synaptic integration of adult-born GCs in the OB is regulated by GABAergic input, as shown by conditional deletion of *Gabra2* to inactivate the main $GABA_AR$ subtype expressed in GCs (Pallotto et al., 2012). Because the absence of these receptors also affects the postsynaptic clustering of gephyrin, we investigated here whether the postsynaptic scaffold organized by gephyrin might contribute to the effects induced by deletion of *Gabra2*.

For this purpose, we generated LVs encoding either rat gephyrin N-terminally fused to eGFP (eGFP-Geph), a phospho-deficient eGFP-gephyrin mutant, known to facilitate postsynaptic gephyrin clustering *in vitro* (eGFP-Geph(S270A)), or a dominant-negative mutant unable to form postsynaptic clusters (eGFP-Geph(L2B)) (see Introduction). We injected these LVs separately into the RMS of adult C57Bl6/J mice and, as control, LV constructs expressing only eGFP or tdTomato. The eGFP fused to each gephyrin isoform allowed us to detect transfected GCs in the OB and to monitor their morphological development by fluorescence microscopy. Based on previous work (Whitman and Greer, 2007, Panzanelli et al., 2009, Pallotto et al., 2012), we decided to follow their maturation between 7 and 90 dpi. For each gephyrin construct and time-point, tissue from 3-6 mice was used for analysis.

Figure 1 Morphology of adult-born GCs transduced by eGFP-gephyrin lentiviruses
A-A". Representative images of olfactory adult-born GCs after 21 dpi expressing eGFP (**A**), eGFP-Geph (A') and eGFP-Geph(S270A) (A") seen by confocal laser scanning microscopy. Over-expression of eGFP-gephyrin causes its aggregation but does not allow visualizing cell morphology. ***B-C.*** Low-magnification images showing the distribution and morphology of tdTomato- and eGFP-Geph(S270A)- or eGFP-Geph(L2B)-positive adult-born GCs at 14 dpi, as detected by immunofluorescence staining against GFP. While tdTomato-transduced cells are much more numerous, no obvious differences in distribution were observed. However, the apical dendrites of eGFP-Geph(L2B)-positive adult-born GCs appear shorter and do not yet reach the EPL. ***D-E.*** Representative images of a double-transduced adult-born GCs at 21 dpi with LVs encoding tdTomato (red) and eGFP-Geph(S270A) (green) or eGFP-Geph(L2B) (green), demonstrating that in both cases the eGFP-tagged proteins can be detected by immunofluorescence in the entire cell (yellow in **D"** and **E"**). Note the paucity of spines in the GC transduced with eGFP-Geph(L2B). ***F-F''.*** Example of an eGFP-Geph-positive GC (green) filled with Lucifer Yellow (red) at 21 dpi, showing double-labeling of dendritic shaft and spines (arrow), whereas filopodia contain no detectable eGFP-Geph immunofluorescence (stars). Scale bars: ***A, D-F,*** 10 μm; ***B-C,*** 100 μm.


A
eGFP
A'
eGFP-Geph
A''
eGFP-Geph(S270A)
D
eGFP-Geph(S270A)
D'
D''
E
eGFP-Geph(L2B)
E'
E''
RMS
GCL
MCL
EPL
B
B'
eGFP-Geph(S270A)
RMS
GCL
MCL
EPL
C
C'
eGFP-Geph(L2B)
F
F'
F''

A first visual inspection of sections from virus-injected mice at early time-points revealed the presence of bright eGFP-Geph clusters in the GCL and EPL; however, it was difficult to trace the morphology of eGFP-Geph infected dendrites compared to the GFP control GCs, which exhibited a uniform dendritic labeling (Fig. 1A-A''). GCs expressing eGFP-Geph(L2B) showed a weak, often undetectable fluorescence (data not shown). Hence, we enhanced the GFP signal in all subsequent experiments using immunohistochemistry allowing for better visualization of the cell soma, dendrites, and spines of transduced adult-born CGs. To verify that the different eGFP-gephyrin isoforms fill up the entire GC, we co-injected a 1:1 mixture of two LVs encoding either eGFP-Geph(S270A) or eGFP-Geph(L2B) along with a LV expressing the red fluorescent protein tdTomato. At low magnification, we observed that GCs expressing eGFPGeph(S270A) (signal enhanced by immunofluorescence) exhibit a similar morphological development at 14 dpi compared to tdTomato-control cells (Fig. 1B,B'), although they were distinctly less numerous, reflecting the difference in titer between the two LVs. Both cell populations had a branched apical dendrite penetrating the EPL. In contrast, GCs expressing eGFP-Geph(L2B) appeared different, having in most cases an unbranched apical dendrite that did not yet reach the EPL (Fig. 1C,C').

Examination of double positive cells (eGFP/tdTomato) at higher magnification revealed that both eGFP-Geph(S270A) and eGFP-Geph(L2B) filled up GCs to the same extent as tdTomato, allowing us to assess their morphology in detail (Fig. 1D-E). Over-expression of the dominant-negative gephyrin isoform caused severe morphological alterations compared to the other LV constructs (Fig.1E-E''), confirming the impression from low magnification images. In particular, it became clear that these cells have a profound deficit in spine formation.

As an additional control, OB slices containing eGFP-Geph-positive GCs were prepared for iontophoretic filling of selected GCs with Lucifer Yellow, followed by double immunofluorescence for GFP and Lucifer Yellow. Examination of double-labeled cells revealed a very strong Lucifer Yellow immunostaining and confirmed that eGFP-Geph fills most spines, but not filopodia, which were systematically single-labeled for Lucifer Yellow only (Fig.1F-F'').

Taken together, these observations revealed that although the eGFP signal had to be amplified by immunolabeling for morphological analysis of transduced adult-born GCs, eGFP-gephyrin is not restricted to postsynaptic sites but fills the entire cell, except for filopodia. As GCs are axonless, we could not determine whether eGFP-gephyrin would also be transported into this subcellular compartment.

Postsynaptic clustering of eGFP-gephyrin

Next, we focused our analysis on the apical dendrite of GCs, which undergoes marked morphological differentiation and is functionally subdivided into two distinct compartments (Shepherd et al., 2007). In the GCL, it receives mainly axo-dendritic inputs from principal cells (onto spines) and local interneurons (on spines and shaft), as well as centrifugal inputs from various brain regions. In the EPL, the apical dendrite branches profusely; its spines form reciprocal, dendro-dendritic synapses with the dendrites of principal cells, whereas its shafts receive GABAergic synapses from local interneurons (short-axon cells).

To determine whether the bright eGFP-positive clusters in adult-born GCs seen after viral injection of either eGFP-Geph or eGFP-Geph(S270A) (Fig. 1A', A'') are postsynaptic gephyrin aggregates, we used false color mapping to better discern the fluorescence intensity differences between diffuse GFP and postsynaptic clusters. Images were acquired such as to saturate the brightest eGFP signals in the dendrites and were displayed using an 8-bit (256 levels) color scale and marking pixels exceeding a gray value of 232 in yellow. As expected, in GCs expressing only eGFP, the diffuse fluorescence was saturated (yellow) in almost the whole cell (Fig. 2A); whereas in GCs expressing either eGFP-Geph or eGFP-Geph(S270A), isolated yellow puncta were observed (Fig. 2B,C), distributed over the dendrites and in spines. Finally, in GCs transduced with eGFP-Geph(L2B), only few saturated clusters were seen in the dendrite, and diffuse eGFP staining predominated (Fig. 2D). Saturated pixels over the cell body reflect the high gephyrin concentration compared to the dendrites (Fig. 2D).

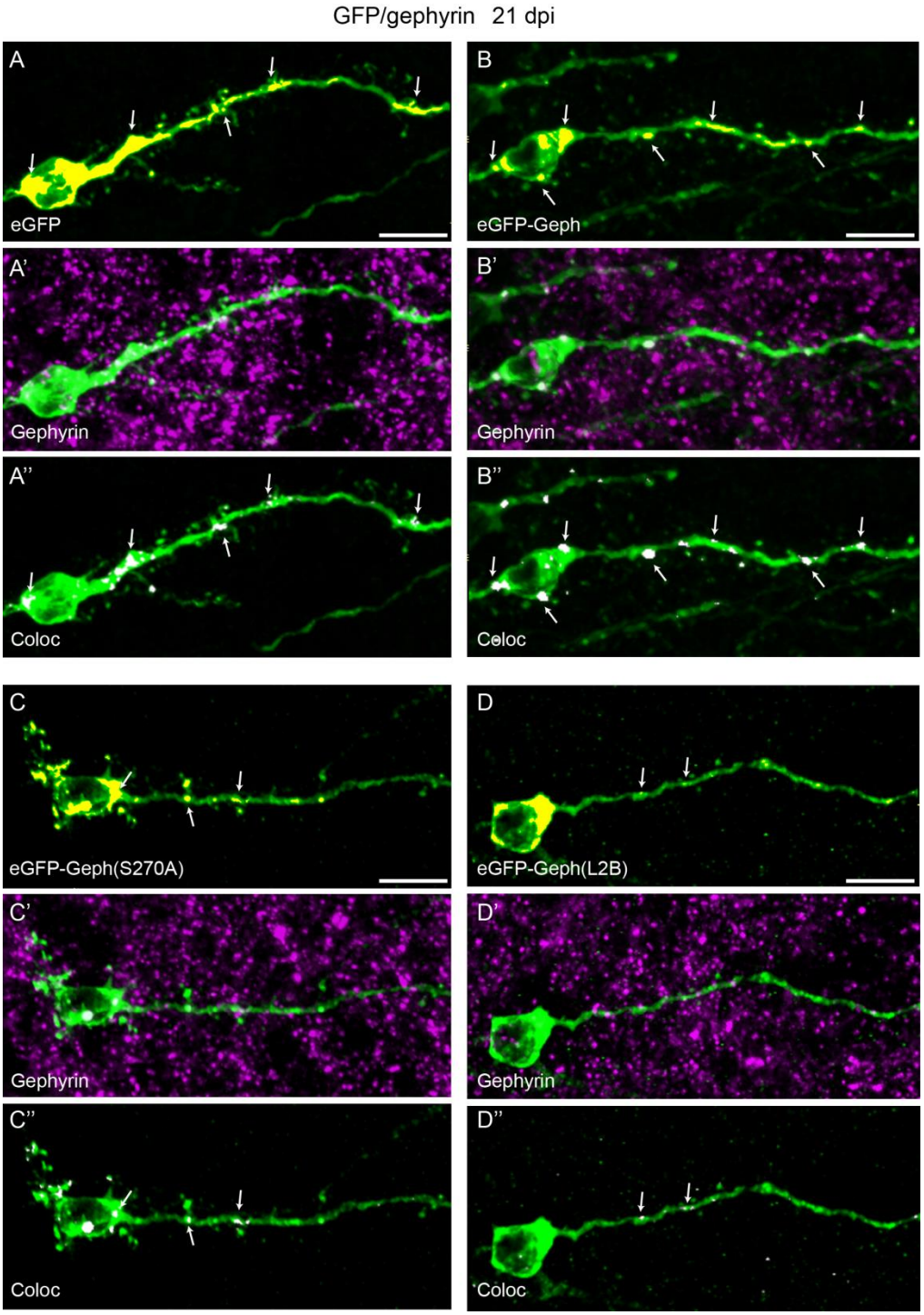


Figure 2 Identification of eGFP-gephyrin clusters as being postsynaptic
A-D. Double-immunofluorescence staining for eGFP and gephyrin (mAb7a; magenta) of OB sections at 21 dpi of the four LV constructs indicated. The upper image in each block displays eGFP immunofluorescence using false color mapping to demonstrate the difference in intensity between diffuse eGFP and clustered eGFP in GC dendrites; images were acquired such as to saturate (yellow) the brightest pixels. The diffuse fluorescence in eGFP-positive GCs (A) shows no local subcellular clustering, whereas in GCs expressing either eGFP-Geph or eGFP-Geph(S270A) the saturated pixels form clusters distributed over the dendrites and spines (B, C). In eGFP-Geph(L2B)-positive GCs, only a few clusters of saturated pixels (D) are present. The middle images (***A'-D'***) display double immunofluorescence of eGFP (green) and gephyrin (mAb7a; magenta) (maximal intensity projection of a stack of 12-15 images spaced by 0.5 µm), depicting the clustered distribution of gephyrin in the OB. Note that the diffuse eGFP-gephyrin is not detected by mAb7a. The lower images (***A''-D''***) show mAb7a-positive clusters colocalized with eGFP-immunofluorescence (white) to demonstrate that they largely coincide with the eGFP saturated pixels shown in A-D, except in GC expression eGFP-only (A). Scale bars: ***A-D,*** 10 µm.

To determine whether the clustered eGFP signals seen in eGFP-Geph- and eGFP-Geph(S270A)-positive GCs are indeed postsynaptic gephyrin aggregates, and whether the different gephyrin isoforms over-expressed in transduced cells interact with endogenous gephyrin, we performed double labeling for GFP and gephyrin (using the monoclonal mAb7a, see Table 1). Most of the eGFP clusters in eGFP-Geph-positive GCs were recognized by this antibody (Fig. 2B', B''; arrows) and the density of gephyrin clusters was comparable to that seen in GCs expressing eGFP (Fig. 2A', A''). In contrast, only a fraction of the bright eGFP clusters present in eGFP-Geph(S270A)-positive GCs were labeled by mAb7a, in line with *in vitro* studies that a conformational change of the Geph(S270A) mutant alters the epitope recognized by this antibody (Tyagarajan et al., 2011b, Kuhse et al., 2012) (Fig. 2C, C', C''). Nevertheless, we infer from the distribution of these eGFP-Geph(S270A)-positive clusters that they represent postsynaptic aggregates. Finally, as expected in GCs expressing eGFP-Geph(L2B), almost no clusters positive for mAb7a were detected (Fig. 2D',D''); confirming previous studies in primary neuronal culture that this construct exerts a dominant-negative effect on endogenous gephyrin clustering (Lardi-Studler et al., 2007).

Taken together, both approaches strongly suggested that the eGFP-tagged gephyrin constructs are able to form postsynaptic clusters along with endogenous gephyrin, except in the case of the L2B mutant. Further, these data show that mAb7a specifically recognizes clustered gephyrin, but not diffusely distributed gephyrin upon over-expression, opening the possibility that a fraction of endogenous gephyrin is also present as non-aggregated molecules in neurons (and possibly non-neuronal cells).

Differential effect on neuronal survival

To determine whether the over-expression of gephyrin mutants, notably Geph(L2B), might affect the survival of adult-born GCs, we used a virus expressing tdTomato as an internal control, and quantified the ratio of green/red cells at 14 and 90 dpi in mice co-injected with two LVs (N=5-7 mice/group). In line with the higher titer of the tdTomato LV compared to the eGFP-Geph(S270A) and eGFP-Geph(L2B) LVs, more red than green cells were observed. On average, about 47-50% of green cells were double-labeled (i.e., double-transduced) in each of the four groups analyzed.

Quantification of tdTomato-positive GCs revealed a large increase in cell number between 14 and 90 dpi (Fig. 3A), suggesting that the LV continuously transduces neuronal progenitors migrating through the RMS and that the population of tdTomato-positive GCs seen at 90 dpi is not of uniform age. The same observation was made by quantifying eGFP-Geph(S270A) and

eGFP-Geph(L2B)-positive GCs at 14 and 90 dpi, although both LVs labeled fewer cells than the tdTomato-LV (Fig. 3A), in line with their lower titer (see Materials and Methods). Therefore, migrating neuronal progenitors were continuously transduced over weeks after LV injection. Importantly, the time-dependent increase eGFP-Geph(S270A)-positive cells was proportionally the same as for tdTomato-positive GCs at 90 dpi (>3-fold), indicating that their net survival rate was similar (Fig. 3A). Therefore, over-expression of this gephyrin construct capable of forming postsynaptic clusters has no impact on long-term survival of adult-born GCs. Interestingly, eGFP-Geph(L2B) infected GCs showed a reduced time-dependent increase in cell number (<2-fold) compared to either tdTomato- or eGFP-Geph(S270A)-positive GCs. The dominant-negative L2B gephyrin mutant might therefore be curtailing the survival of adult-born GCs.

To further explore the mechanisms underlying the reduced survival of GCs expressing eGFP-Geph(L2B) compared to eGFP-Geph(S270A) or tdTomato, we modeled survival curves based on the following assumptions: 1) The number of newborn GCs reaching the OB depends on the titer/infectivity of each LV, and declines gradually (e.g., 5% per week) independently of LV injected; 2) the mortality rate of newborn GCs is initially high and declines exponentially during the first 3 weeks with a fixed time constant (e.g., 20% per week; explaining the rapid elimination of the majority of adult-born GCs that fail to differentiate); 3) The mortality rate of GCs that survive post-3 weeks subsequently rises slowly (e.g., 3% per week; to account for the limited life-span of adult-born GCs (Ninkovic et al., 2007)). We used the model to calculate the number of labeled GCs present weekly in OB, selecting the mortality rates and time-constants to fit with our actual countings at 14 and 90 dpi (see details in the legend of Fig. 3A-E).

A

	Injection of LV mixture tdTomato + eGFP-Geph(S270A)			Injection of LV mixture tdTomato + eGFP-Geph(L2B)		
	tdTomato	eGFP-Geph(S270A)	Percentage of eGFP-Geph(S270A)	tdTomato	eGFP-Geph(L2B)	Percentage of eGFP-Geph(L2B)
14 dpi	279±30	48±9	16.1	293±26	85±12	26.2
90 dpi	1057±114	165±17	14.6	1009±108	153±18	14.1

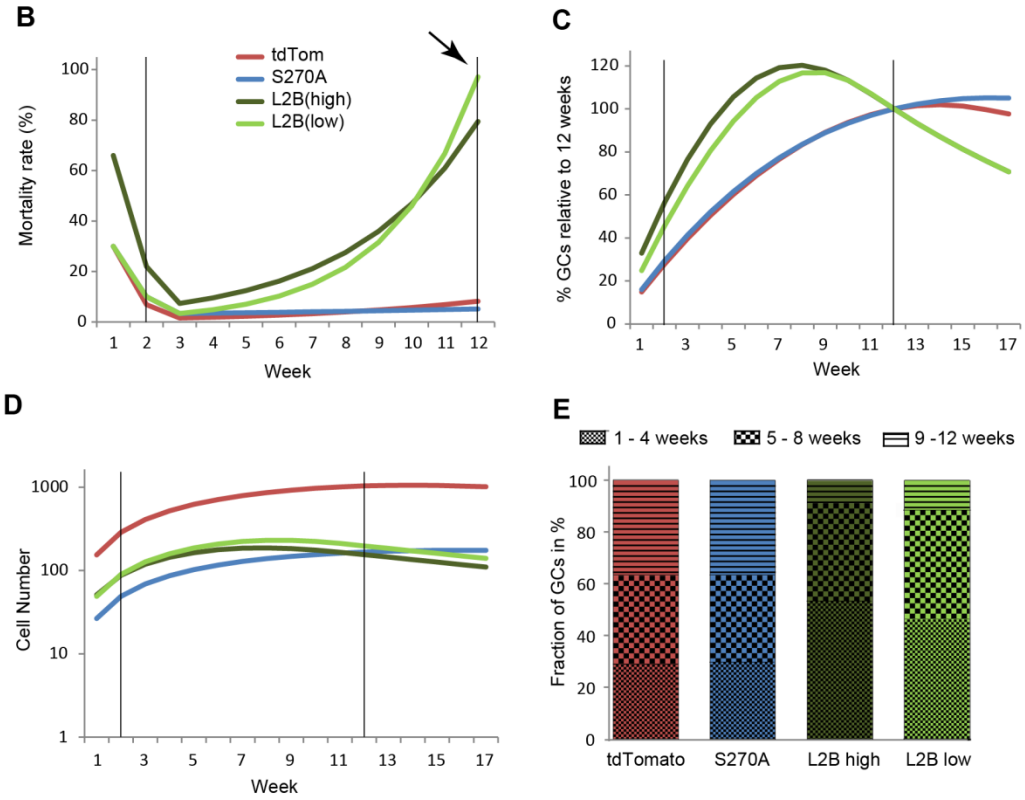


Figure 3 Effect of eGFP-Geph(S270A) and eGFP-Geph(L2B) over-expression on neuronal survival
A. Number of GCs (mean ± SEM; *N*=5-7) positive for tdTomato, eGFP-Geph(S270A) and eGFP-Geph(L2B) per 50 µm-thick coronal section at 14 and 90 dpi following injection of a mixture of tdTomato and eGFP-gephyrin LVs. The proportion of eGFP-/tdTomato-positive cells was calculated at each time-point. It was similar for GCs transduced by eGFP-Geph(S270A) but lower for eGFP-Geph(L2B)-positive GCs, suggesting increased mortality. ***B-E.*** Modeling data testing whether GCs transduced by eGFP-Geph(L2B) have a higher mortality rate initially (i.e. weeks 1-3, before differentiation) or late (i.e., weeks 4-12, after differentiation), or both. The model assumes three conditions: 1) continuous (but slowly declining) production of transduced GCs; 2) initially high, but rapidly declining mortality rate during weeks 1-3; 3) long-term low, but slowly raising mortality rate thereafter. Panel ***B*** shows mortality rate constants for tdTomato-positive GCs required to reach the number of counted cells at 14 and 90 dpi (vertical bars); nearly identical time-constants apply for eGFP-Geph(S270A)-positive GCs. For eGFP-Geph(L2B)-positive cells, two scenario are depicted ($L2B_{low}$: initial mortality rate identical to tdTomato (33% per week); $L2B_{high}$: initial mortality rate doubled; in both cases, the late mortality rates have to be much higher than for tdTomato to reach the cell numbers seen at 90 dpi, suggesting that the eGFP-Geph(L2B) LV limits the life-span of adult-born GCs. However, scenario $L2B_{low}$ is unlikely, because the late mortality rate reaches 100% at 12 weeks post-injection (arrow); therefore, this gephyrin mutant likely also affects the survival of non-differentiated GCs. Panels ***C*** and ***D*** depict the numbers of labeled GCs expected weekly based on the mortality rates shown in ***A***. For tdTomato and eGFP-Geph(S270A), the maximum is reached after 3 months, whereas for eGFP-Geph(L2B) it is reached at either 7.5 or 8.5 weeks; these data also allowed calculating the average life-span of GCs that had survived the first 3 weeks (see main text). Panel **E** depicts the age-distribution of adult-born GCs at 90 dpi, depending on their mortality rate. The number of GCs produced after the first week had to be set to 220 for tdTomato, 38 for eGFP-Geph(S270A), 70 for eGFP-Geph$(L2B)_{low}$, and 150 for eGFP-Geph$(L2B)_{high}$. A weekly rate of decline in production was set identically for all constructs (5%). Changing these parameters had little impact on the differential shape of the curves shown in panels B-D, but imposed to adjust the mortality rates accordingly.

The model shows that both the early and late mortality rate constants of tdTomato- and eGFP-Geph(S270A)-expressing GCs can be set to nearly identical values, assuming, however, that the production rate of tdTomato-positive GCs is about 6 times higher than that of eGFP-Geph(S270A)-positive GCs (Fig. 3B; blue and red curves). One can extrapolate from the time-constant of the late mortality rate and derive that the longevity of those GCs that have survived up to 3 weeks is 17 weeks. In contrast, the reduced fraction of eGFP-Geph(L2B)-positive GCs at 90 dpi can be explained using the best fit model obtained by doubling the higher early mortality rate and by increasing the late mortality rate constant 3-fold (Fig. 3B-C; $L2B_{high}$, dark-green curves). According to these parameters, the longevity of eGFP-Geph(L2B)-positive GCs does not exceed 13 weeks. Keeping the early mortality rate unchanged compared to the tdTomato LV (33%) is also feasible, but it requires such a high late mortality rate constant that the longevity of GCs would be less than 10 weeks (Fig. 3B-C; $L2B_{low}$, light-green curves), which we considered to be unlikely. Nevertheless, these examples showed that the model is robust and the outcome after 12 weeks can be very similar, independently of the initial mortality rate (Fig. 3B-C; $L2B_{high}$ versus $L2B_{low}$). When calculating the total number of adult-born GCs present at the end of each of the 12 weeks analyzed, the model revealed that the maximum is reached at 7-8 weeks post-injection for eGFP-Geph(L2B)-positive GCs and at 14 weeks for tdTomato- and eGFP-Geph(S270A)-positive GCs (Fig. 3D). Finally, the differences in longevity predicted from this model affect the age distribution of adult-born GCs analyzed at 12 weeks post-injection (Fig. 3E): in mice injected with tdTomato or eGFP-Geph(S270A) LV, the age groups 1-4 weeks, 5-8 weeks, and 9-12 weeks are roughly equal, with >35% of cells being in the oldest group. In mice injected with eGFP-Geph(L2B) LV, the oldest cells make up about 10% of the total population, and the youngest account for nearly 50%. Altogether, these results and modeled data confirm that over-expression of a gephyrin construct that precludes its postsynaptic clustering is detrimental for survival of adult-born GCs.

In these experiments, we also measured radial migration of adult-born GCs away from the RMS to determine whether this parameter would be influenced by gephyrin mutations. The migration distance was measured at 14 and 90 dpi, both as the absolute radial distance from the outer border of the RMS to the cell center and as the proportional distance within the GCL (whose thickness was determined from the outer border of the RMS to inner border of the mitral cell layer). The results are given in Table 2. No significant difference was detected among the three LVs analyzed at either 14 or 90 dpi, but a significant effect of time (eGFP(S270A): $F_{(1,12)} = 42.57$; $P<0.0001$; eGFP(L2B): $F_{(1,14)} = 72.37$, $P<0.0001$) was observed, indicating that migration into the GCL continues for several weeks without being influenced by gephyrin over-expression.

This conclusion is most evident when considering the migration distance relative to the width of the GCL; the fraction covered by migrating GCs increased by 25% from 40% at 14 dpi to 50% of the total GCL width at 90 dpi (Table 2).

Table 2: **Radial migration of adult-born GCs**

Injection of LV mixture tdTomato + eGFPGeph(S270A)

	Absolute radial migration (μm)		Migration in % GCL width	
	tdTomato	eGFP-Geph(S270A)	tdTomato	eGFP-Geph(S270A)
14 dpi	121±9	118±10	43	39
90 dpi	151±10	151±10	50	49

Injection of LV mixture tdTomato + eGFP-Geph(L2B)

	Absolute radial migration (μm)		Migration in % GCL width	
	tdTomato	eGFP-Geph(L2B)	tdTomato	eGFP-Geph(L2B)
14 dpi	121±9	113±9	42	38
90 dpi	149±9	147±9	51	50

Differential alteration in dendrite development

We hypothesized that the reduction in dendritic arbor complexity after conditional deletion of α2-$GABA_A$Rs in adult-born GCs (Pallotto et al., 2012) might be due to the disruption of the GABAergic postsynaptic density and its associated signaling molecules, thereby affecting neuronal maturation. To substantiate this hypothesis, we quantified major morphometric parameters in GCs expressing eGFP-gephyrin (Fig. 4A-C). Quantification of number of terminal branches and total dendritic length revealed a significant effect of time ($F_{Branches(2.426)}$ = 6.833, P = 0.0012; $F_{Length(2.450)}$ = 14.47, P<0.0001) and gephyrin construct ($F_{Branches(3.426)}$ = 102.9, P<0.001; $F_{Length(3.450)}$ = 125.5, P<0.0001), but no interaction. *Post-hoc* analysis showed that eGFP-Geph(S270A) increases the number of terminal branches compared to eGFP-Geph at 21

and 90 dpi, without affecting total dendritic length, whereas eGFP-Geph(L2B) over-expression significantly reduces dendritic branching and length at all time-points (Fig. 4A-B).

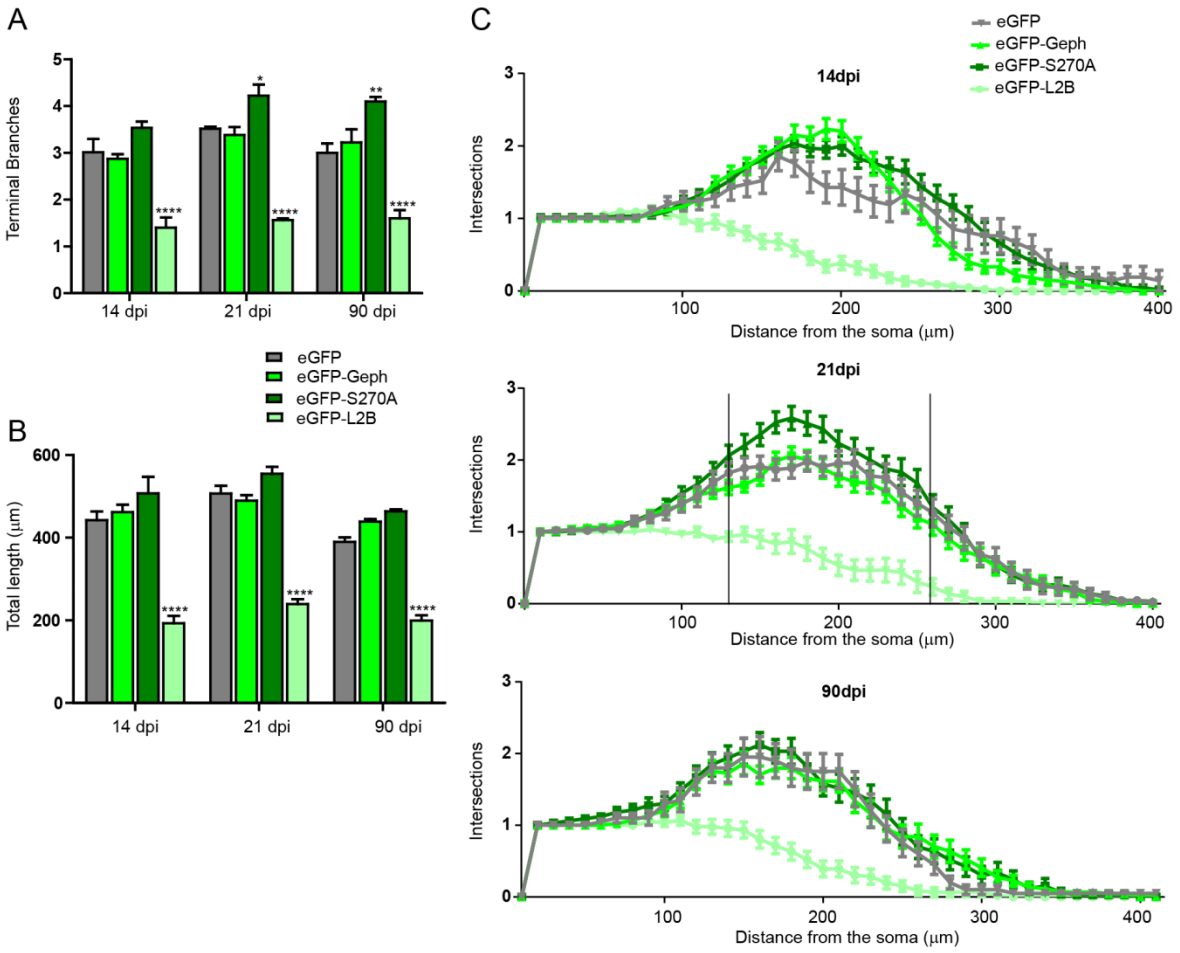


Figure 4 Morphometric analysis of apical dendrite arborization of eGFP-gephyrin-transduced GCs
A-B. Quantification of terminal branches and total dendritic length, represented by bar-graphs (mean ± SEM; *N*=3-6 mice/group). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 compared to eGFP and eGFP-Geph; Tukey *post-hoc* tests). No significant difference over time was observed. ***C.*** Quantification of apical dendritic arborization by Sholl analysis at 14, 21 and 90 dpi. The number of intersections between eGFP-positive dendrite segments and virtual concentric lines centered on the cell body and spaced by 10 µm is depicted (mean ± SEM; *N*=3-6 mice/group). For statistical comparison between groups, the area-under-the-curve was compared by one-way ANOVA at each time-point. No difference in dendritic complexity was observed, except for GCs expressing eGFP-Geph(L2B) (*P*<0.001 at each time-point). In addition, at 21 dpi, quantification of eGFP-Geph(S270A)-positive dendrites in the inner half of the EPL (between the two vertical lines) revealed a significant difference compared to eGFP and eGFP-Geph (*P*<0.05).

In addition, using Sholl analysis to quantify dendritic complexity, we observed that GCs expressing eGFP-Geph were similar to those expressing eGFP-only at each time-point analyzed (Fig. 4C), whereas GCs expressing eGFP-Geph(L2B) were much shorter and less complex, confirming the morphological deficits observed upon visual inspection (Fig. 2D) (one-way ANOVA; 14 dpi: $F_{(3,160)} = 9.175$, $P<0.0001$; 21 dpi: $F_{(3,160)} = 8.942$, $P<0.0001$; 90 dpi: $F_{(3,160)} = 4.930$, $P<0.01$). The phospho-deficient eGFP-Geph(S270A) mutant, which favors the formation of GABAergic synapses *in vitro*, modulated dendritic development in adult-born GCs. Although Sholl analysis revealed no significant overall differences compared to control cells at any time-point (Fig. 4C), their dendritic arborization was more complex when entering the EPL (on average, between 130 and 260 μm from the soma) at 21 dpi (Fig. 4C) (one-way ANOVA, $F_{(3,64)} = 57.94$, $P<0.0001$).

Taken together, these results reinforce the notion that the postsynaptic clustering properties of gephyrin are linked with neuronal differentiation and that proper formation of gephyrin clusters is a prerequisite for normal dendritic arborization, as shown *in vitro* (Rui et al., 2013).

Gephyrin-dependent modulation of spine formation

To further explore the effects of gephyrin on neuronal maturation, we next investigated how it influences spine formation on the apical dendrites of adult-born GCs. In mature GCs, it is well established that spines located on dendrites within the GCL are innervated by glutamatergic terminals, whereas those located in the EPL form reciprocal synapses with the dendrites of principal cells. In addition, we have shown previously that in immature GCs, at 3-7 dpi, GABAergic inputs onto the apical dendrite are formed transiently on spines, whereas in mature cells the vast majority of GABAergic synapses are located on the shaft (Panzanelli et al., 2009). Accordingly it is conceivable that gephyrin modulates spine formation, as we observed here that the various eGFP-gephyrin constructs tested also labeled spines in both immature and mature adult-born GCs.

In the GCL, quantification of spine density at 14, 21 and 90 dpi revealed a significant effect of time ($F_{(2.395)} = 7.044$, $P = 0.001$) and gephyrin construct ($F_{(3.395)} = 80.16$, $P<0.0001$), but no interaction ($F_{(6.395)} = 0.3620$, $P = 0.9027$). *Post-hoc* analysis showed no significant differences in GCs expressing either eGFP or eGFP-Geph (Fig. 5A, B), suggesting that over-expression of eGFP-Geph does not interfere with spine formation. In contrast, GCs expressing eGFP-Geph(S270A) exhibited a significant increase in spine density persisting up to 90 dpi (Fig. 5A,B), but without significant changes over time (Fig. 5B). This result suggested that eGFP-Geph(S270A) facilitates spine growth; however, because it is puzzling that gephyrin clusters

persist in spines of mature GCs, it is likely that this construct favors the continuous formation of immature, short-lived spines receiving GABAergic, rather than glutamatergic input.

Finally, in line with its detrimental effects on cell survival and dendrite formation, over-expression of eGFP-Geph(L2B) induced a clear deficit in spines on apical dendrites in the GCL (Fig. 5A), evident at each of the time-points analyzed compared to control (Fig. 5B).

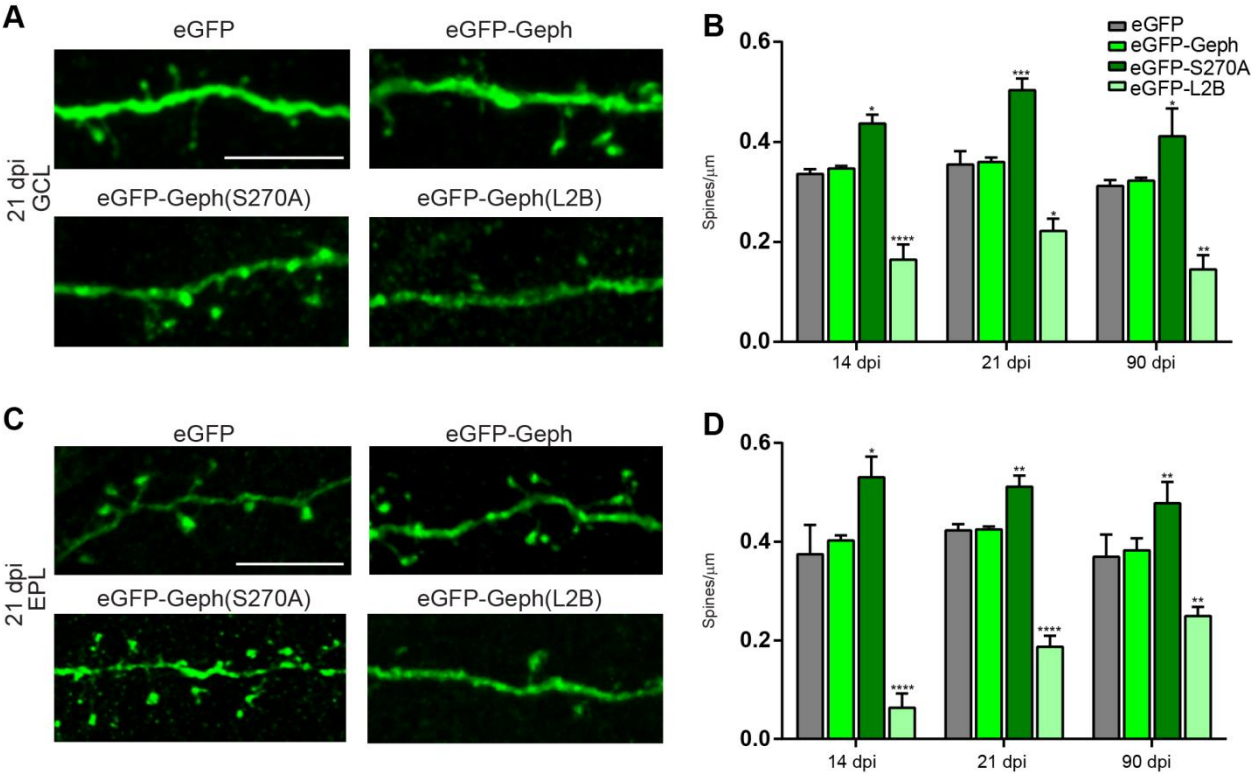


Figure 5 Quantification of spine density of adult-born GCs in the GCL and EPL
A, C. Representative images of spines on the apical dendrite of eGFP-positive GCs in the GCL (**A**) and EPL (**C**) at 21 dpi, illustrating that the majority of immuno-positive spines have a mature morphology and contain a prominent eGFP-gephyrin aggregate, except for eGFP-Geph(L2B). ***B, D.*** Quantification of spine density (mean ± SEM; N=3-6 mice/group) on apical dendrites in the GCL (B) and EPL (D). No significant difference was observed between eGFP- and eGFP-Geph-positive GC at all time-points. eGFP-Geph(S270A)-positive GCs showed a significant increased spine density, whereas eGFP-Geph(L2B)-positive GCs had a reduced spine density (**P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001 compared to eGFP and eGFP-Geph; Tukey *post-hoc* test). Scale bars: ***A, C,*** 10 µm.

In the EPL, quantification of spine density also revealed a significant effect of time ($F_{(2.418)}$ = 4.982, P = 0.0073) and gephyrin construct ($F_{(3.418)}$ = 126.9, P<0.0001), as wells as a significant interaction ($F_{(6.418)}$ = 5.704, P<0.0001). *Post-hoc* analysis showed that over-expression of eGFP-Geph again had no effect on spine density compared to eGFP (Fig. 5C, D), whereas eGFP-Geph(S270A) caused a net increase in spine density at all time-points (Fig. 5C, D). Expectedly, eGFP-Geph(L2B) expression had a strong negative effect in the EPL as seen in the GCL,

although a gradual increase in spine density was detected over time (Fig. 5C, D). Taking into account that these cells might have a limited time-span and that the majority of cells seen at 90 dpi are actually younger (see Fig. 3E), these results suggest that GCs expressing eGFP-Geph(L2B) are able eventually to form reciprocal synapses with mitral cell dendrites, while being strongly impaired to form transient GABAergic synapses on spines.

Presence of postsynaptic α2-$GABA_A$Rs on spines

As noted above, the presence of clustered gephyrin in a substantial fraction of spines in GCs expressing eGFP-geph (and a majority of those expressing eGFP-Geph(S270A); see Fig. 2) was intriguing, especially at 90 dpi, when most cells are mature. To determine whether these spines are indeed postsynaptic to GABAergic input (rather than forming glutamatergic synapses in the GCL and reciprocal synapses in the EPL), we performed triple immunofluorescence with antibodies against GFP, $GABA_A$R α2 subunit, and vesicular GABA transporter (vGAT; to label presynaptic terminals). These experiments also allowed investigating how gephyrin influences the synaptic integration of adult-born GCs by quantifying presumptive GABAergic synapses formed on their apical dendrites. In particular, we wanted to verify the assumption that the excess of spines, seen in dendrites of eGFP-Geph(S270A)-positive GCs in the GCL as well as EPL, reflects recently formed immature spines containing α2-$GABA_A$Rs.

Analysis of α2-subunit cluster density was done separately on the shaft and spines of apical dendrites in the GCL (up to the first bifurcation, typically located close to the mitral cell layer), as well as in the EPL at 14, 21 and 90 dpi. The presence of α2-subunit cluster was determined by colocalization of red (α2) and green (eGFP) immunofluorescence signals. The results are expressed in density (number of clusters/μm) in Figure 6 and given as total cluster number per dendrite in Table 3.

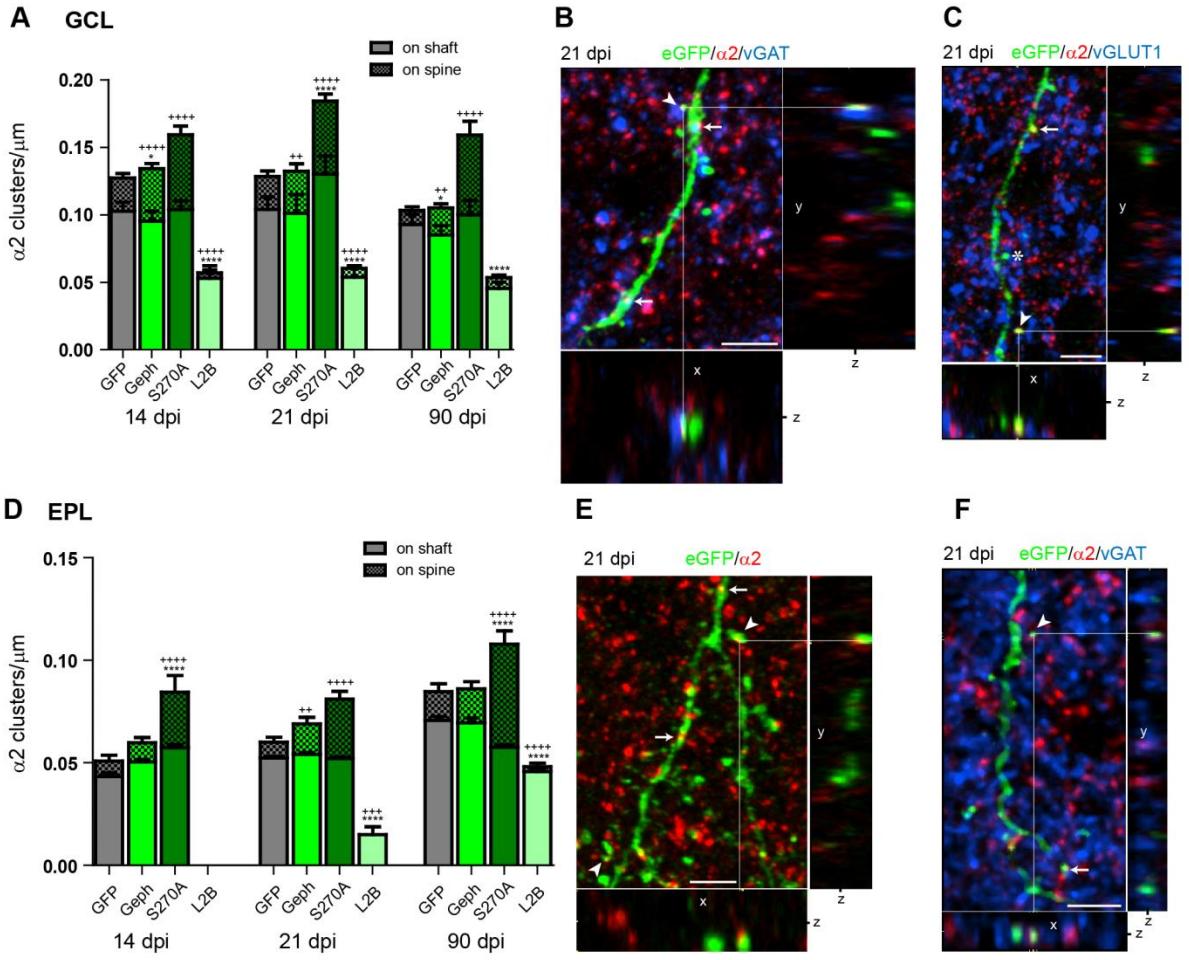


Figure 6 Synaptic integration of adult-born GCs in the GCL and EPL
A, D. Quantification of the density of α2-subunit clusters co-localized with eGFP on shaft and spines at 14, 21 and 90 dpi in the GCL (**A**) and EPL (**D**) (mean ± SEM; *N*=3-6 mice/group) (*,$^{+}$*P*<0.05; **,$^{++}$*P*<0.01; ***,$^{+++}$*P*<0.001 ; ****,$^{++++}$*P*<0.0001 compared to eGFP; Tukey *post-hoc* test; shaft (*); spine ($^{+}$)). Comparison of these data with Fig. 5B, D reveals that the fraction of spines carrying an α2-subunit cluster is much lower in the EPL than GCL, in line with the predominance of reciprocal synapses on spines in the EPL. ***B.*** Triple immunofluorescence staining (3D projections depicted in the 3 Cartesian planes) for eGFP (green), α2-subunit (red) and vGAT (blue), depicting α2-subunit clusters on eGFP-Geph(S270A)-positive dendritic shafts (arrow) and spines (arrowhead) apposed to vGAT-immunofluorescent terminals in the GCL at 21 dpi. ***C.*** Triple immunofluorescence staining for eGFP (green), α2-subunit (red) and vGLUT1 (blue) showing that α2-subunit clusters in eGFP-Geph(S270A)-positive GCs dendritic shafts (arrow) and spines (arrowhead) are not apposed to vGLUT1-immunofluorescent terminals; whereas spines negative for the α2-subunit were often apposed to vGLUT1 terminals (stars). ***E, F.*** Double and immunofluorescence staining for eGFP (green), α2-subunit (red) and vGAT (blue) depicting α2-subunit clusters on eGFP-Geph(S270A)-positive dendritic shafts (arrow) and spine-like structures (arrowhead) in the EPL at 21 dpi. Scale bars: ***B,C,E,F***, 5 µm.

In the GCL, quantification of the α2-subunit density on dendritic shafts revealed a significant effect of time (two-way ANOVA; $F_{(2.399)}$ = 113.6, *P*<0.0001) and gephyrin construct ($F_{(3.399)}$ = 880.4, *P*<0.0001), as well as a significant interaction ($F_{(6.399)}$ = 17.20, *P*<0.0001). *Post-hoc* tests revealed that eGFP-Geph(L2B)-positive GCs carried much fewer α2-subunit clusters (Fig. 6A-C

and Table 3) compared to all other constructs. In spines, a significant effect of time ($F_{(2.402)}$ = 68.3, P<0.0001) and gephyrin construct ($F_{(3.402)}$ = 2100, P<0.0001), and a significant interaction ($F_{(6.402)}$ = 64.77, P<0.0001) were likewise observed. Further, *post-hoc* analyses confirmed that eGFP-Geph(S270A) expression led to a strong increase (2-5 fold) in α2-subunit-positive clusters, particular at 90 dpi, whereas eGFP-Geph(L2B) had a marked negative effect (Fig. 6A-C and Table 3).

Table 3: Number of α2-subunit clusters on dendrites and spines

GCL

α2-subunit clusters on shaft/ primary segment

	eGFP	eGFP-Geph	eGFP-Geph(S270A)	eGFP-Geph(L2B)
14 dpi	16.0±1.1	12.6±1.9	14.2±1.3	10.0±1.0
21 dpi	14.6±1.7	14.0±2.4	16.3±1.8	8.5±2.6
90 dpi	12.2±3.1	12.0±3.3	10.3±1.3	6.7±1.9

α2-subunit clusters on spines/ primary segment

	eGFP	eGFP-Geph	eGFP-Geph(S270A)	eGFP-Geph(L2B)
14 dpi	3.4±1.6	4.7±1.7	7.0±1.6	0.7±0.5
21 dpi	3.5±0.8	3.8±1.4	6.6±0.9	0.3±0.4
90 dpi	1.5±0.4	2.4±1.3	6.2±2.1	1.1±0.7

EPL

α2-subunit clusters on shaft/ apical dendrites in the EPL

	eGFP	eGFP-Geph	eGFP-Geph(S270A)	eGFP-Geph(L2B)
14 dpi	12.8±5.2	16.1±2.0	19.2±7.9	
21 dpi	21.0±3.3	24.5±4.4	23.9±4.7	2.4±1.2
90 dpi	21.9±1.0	20.7±1.1	19.9±4.8	10.1±2.2

α2-subunit clusters on spines/ apical dendrites in the EPL

	eGFP	eGFP-Geph	eGFP-Geph(S270A)	eGFP-Geph(L2B)
14 dpi	2.2±0.5	3.0±1.2	11.3±9.6	
21 dpi	2.2±2.1	4.1±3.3	13.9±3.5	0.0±0.0
90 dpi	4.3±1.4	5.0±0.5	18.4±1.6	0.7±0.7

As seen in Figure 6A, α2-subunit clusters located in spines represent about 25-30% of the total number of α2 clusters in the apical dendrite within the GCL. We conclude from these results that these spines containing α2-$GABA_A$Rs are probably newly formed, as reported previously in immature GCs at 3-7 dpi (Panzanelli et al., 2009). As a corollary, these results confirm our hypothesis that expression of eGFP-Geph(S270A) favors the formation of immature spines, which are most likely transient (since the density of GABAergic synapses on the shaft does not increase much over time).

In the EPL, the density and number of α2-subunit clusters on dendritic shafts varied little over time and among the constructs analyzed, except for GCs expressing the eGFP-Geph(L2B), which exhibited a remarkable time-dependent increase to reach levels similar to those of the other constructs at 90 dpi (Fig. D-E and Table 3) (two-way ANOVA; $F_{(6.259)} = 41.88$, $P<0.0001$). In spines, two-way ANOVA revealed a significant effect of time ($F_{(2.248)} = 123.3$, $P<0.0001$) and gephyrin construct ($F_{(3.248)} = 731.1$, $P<0.0001$), as well as a significant interaction ($F_{(6.248)} = 32.68$, $P<0.0001$). α2-subunit clusters are unlikely to be present in spines forming reciprocal synapses with mitral cell dendrites. Accordingly, the density of spines containing the α2-subunit was very low, except for eGFP-Geph(S270A)-positive GCs, where it was about 5 times higher than control, representing up to 50% of all α2-subunit clusters present on their dendrites. This remarkable observation reinforces the notion that this gephyrin mutant construct favors the formation of GABAergic synapses on spines, albeit short-lived, as they are not efficiently converted into GABAergic synapses on the shaft. Conversely, expression of eGFP-Geph(L2B) virtually abolished the ability of GCs to form such GABAergic synapses on spines, whereas the formation of α2-subunit-negative spines, likely representing regular reciprocal synapses, was only partially impaired.

Finally, we were wondering whether spines containing the α2-$GABA_A$R are innervated by a presynaptic terminal containing vGAT. Visual inspection of high resolution images revealed that the majority, but not all, spines positive for the α2-subunit were apposed to vGAT clusters; likewise the same observation was seen in the dendritic shaft (Fig. 6B). Quantitative analysis in the GCL confirmed that, on average, 70% of α2-subunit clusters were apposed to presynaptic vGAT clusters. In the EPL, such quantitative analysis was not possible, due to the very high density of vGAT-positive puncta, which mostly represent GC spines forming a reciprocal synapse with mitral cell dendrites. As a control, we verified that spines positive for the α2-subunit were not innervated by terminals containing vGLUT1, a marker of glutamatergic transmission. As expected, we observed in the GCL vGLUT1 labeling typically was apposed to

GFP-positive spines negative for the α2-subunit (Fig. 6C). A similar analysis was not possible in the EPL, again due to the very high density of vGLUT1 immunoreactivity within mitral cell dendrites, presumably forming reciprocal synapses with GC spines.

Ultrastructural features of GCs are not altered by over-expression of eGFP-gephyrin constructs

The results so far revealed that eGFP-gephyrin constructs label all spines of adult-born GCs, either diffusely or by forming a bright fluorescent cluster. While gephyrin clusters in the spines often were co-localized with the α2-subunit, presumably forming transient GABAergic synapses, we wondered whether eGFP-gephyrin is also present in spines receiving glutamatergic synapses or in spines engaged in dendro-dendritic connections in the EPL. Therefore, we used double immuno-labeling electron microscopy (EM) with antibodies against GFP and either gephyrin (mAb7a) or vGAT to analyze the ultrastructure of eGFP-positive spines, and notably the formation of GABAergic and reciprocal synapses. Analysis of GCs transduced with eGFP-Geph showed that the intensity of the GFP immuno-labeling was much stronger in the GCL than EPL, where it was barely detectable. Nevertheless, we readily observed in the GCL the presence of labeled profiles making or receiving a GABAergic synapse (Fig. 7A1-A3), some of which contained a prominent gephyrin immuno-labeling. In the EPL, all eGFP-positive spines observed were either presynaptic to a gephyrin-positive mitral cell profile, or were forming a reciprocal synapse (Fig. 7A4-A5). Despite extensive sampling, we did not observe any postsynaptic GFP-positive profile; in part, this might be due to the weak overall GFP labeling, possibly leading to many false-negative GC spine profiles.

Essentially the same results were obtained in GCs transduced with the eGFP-Geph(S270A) LV (Fig. 7B1-B3), except that some incoming GABAergic synapses in the GCL lacked gephyrin immunolabeling. In the EPL, we again observed in tissue from 6 mice that eGFP-positive profiles were all presynaptic, forming gephyrin-immunoreactive GABAergic synapses with mitral cell profiles, as well as reciprocal synapses (Fig. 7B4-B6).

Finally, no major ultrastructural alteration was seen in GCs expressing eGFP-Geph(L2B). No postsynaptic eGFP-positive profile could be found in either the GCL or EPL (in line with the paucity of synapses received by these cells (Fig. 7C1-C6). Unexpectedly, synapses formed by GFP-positive profiles in the GCL had a rather prominent postsynaptic density, which lacked gephyrin immunolabeling (Fig. 7C1-C2), although gephyrin immunogold labeling was detectable in their vicinity. In the EPL, eGFP-Geph(L2B)-positive profiles formed characteristic GABAergic and reciprocal synapses with mitral cell dendritic profiles, prominently

immunopositive for gephyrin (Fig. 7C5-C6). Altogether, these results confirm that the presence of eGFP-gephyrin in spines cannot be taken as evidence for postsynaptic clusters, and that it does not affect apparently their functional differentiation, notably to establish reciprocal synapses with the dendrites of principal cells.

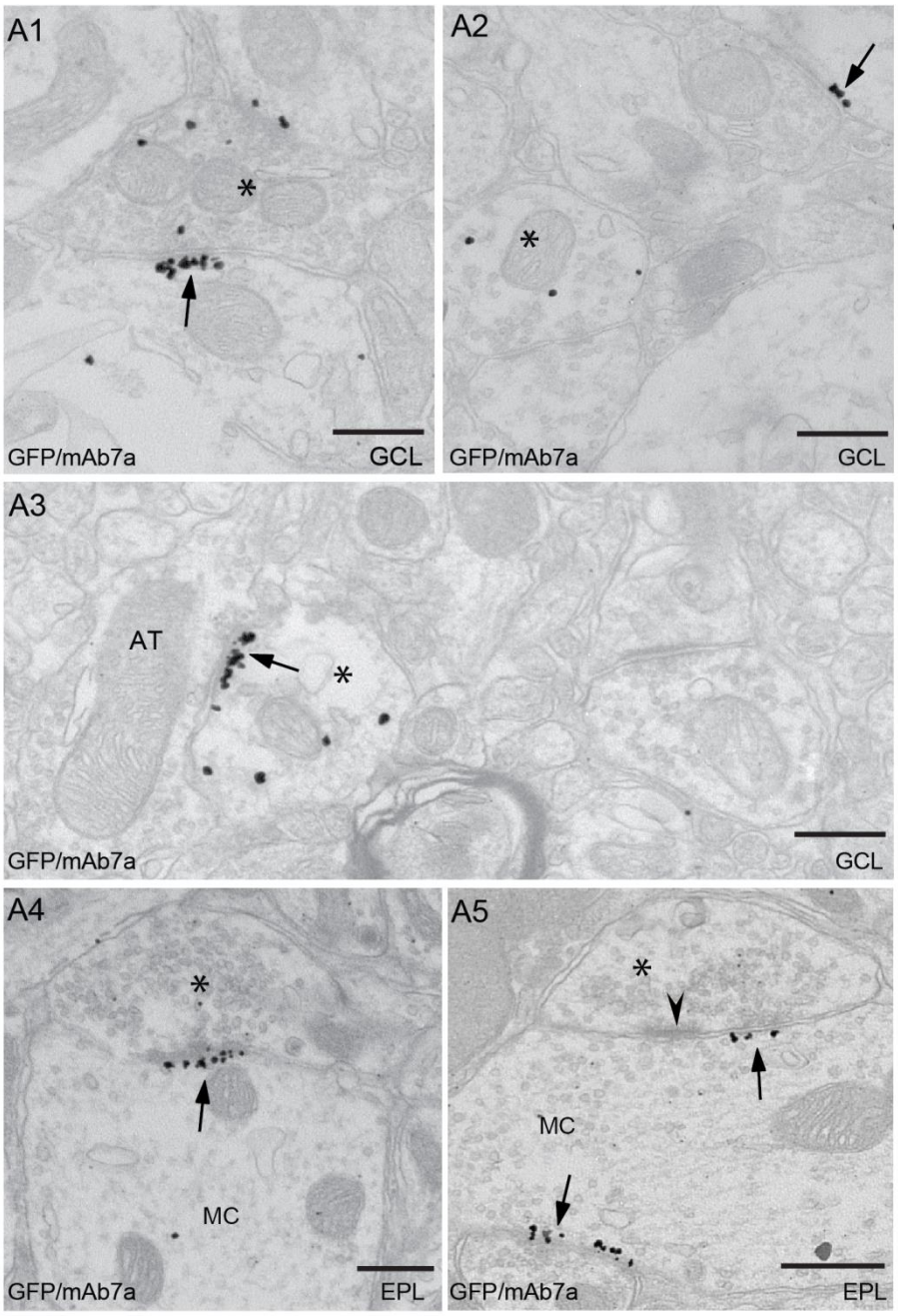


Figure 7*A* Double-labeling immunoelectron microscopy of adult-born GCs expressing eGFP-Geph at 21 dpi. Single- and double-labeled profiles were identified in the GCL and EPL and analyzed for their synaptic contacts. eGFP was detected by immunoperoxidase staining and either gephyrin (mAb7a) or vGAT by immunogold labeling. ***A1-A3***. eGFP-Geph-positive profiles (*) in the GCL making or receiving symmetric synapses that are either strongly labeled for gephyrin (**A1**, **A3**; arrow) or devoid of labeling (**A2**). ***A4-A5*.** Examples of GABAergic and reciprocal synapses between a mitral cell (MC) dendrite and eGFP-positive GC profile (*), as well as an unlabeled profile (A4; bottom left); the arrows point to gephyrin immunogold labeling; the arrowhead indicates the glutamatergic postsynaptic density. Scale bars: 0.5 μm.

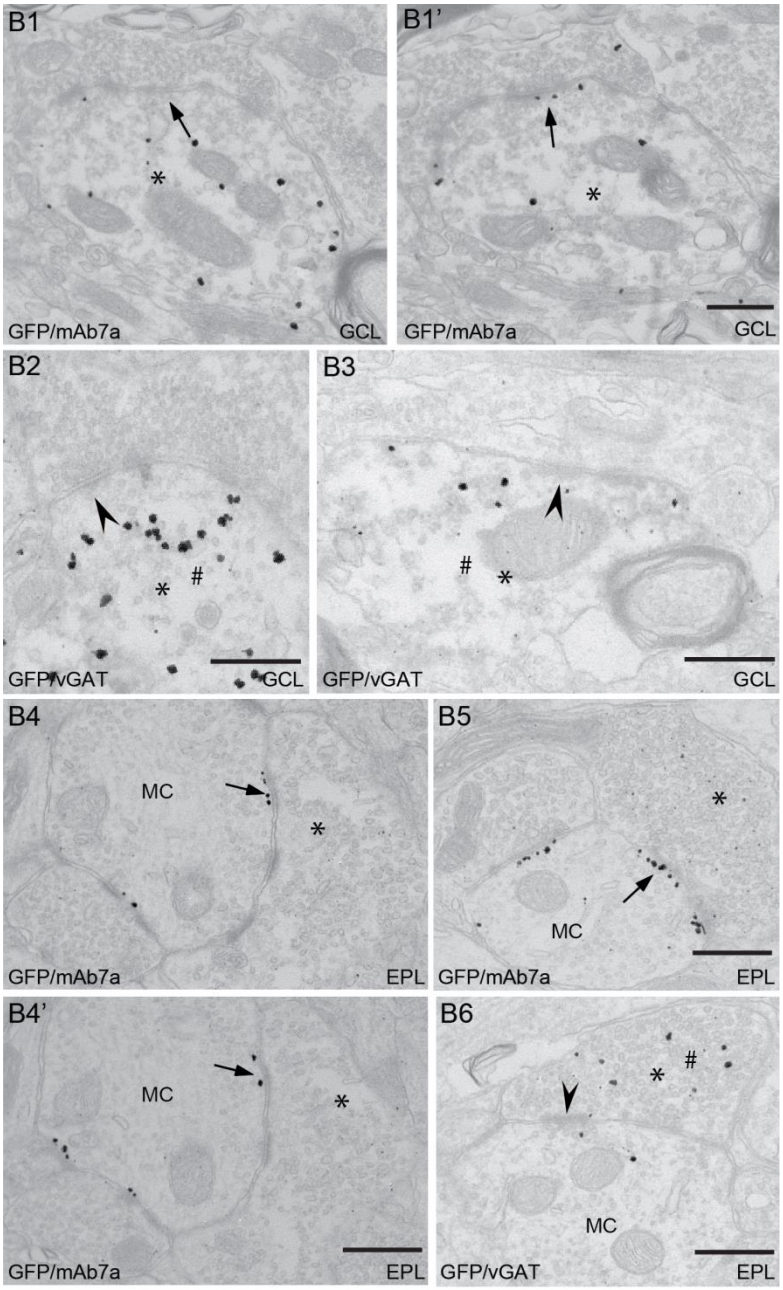


Figure 7*B* Double-labeling immunoelectron microscopy of adult-born GCs expressing eGFP-Geph(S270A) at 21 dpi. *B1-B1'*. Pair of consecutive sections depicting a presynaptic profile in the GCL making multiple symmetric synapses, some of which labeled for gephyrin (arrows) on a GC profile positive for eGFP-Geph(S270A) (*). ***B2-B3***. Double-labeled GC profiles in the GCL [eGFP-Geph(S270A) (*) and vGAT (#)] receiving a symmetric (**B2**) or an asymmetric (**B3**, arrowhead) synapse from immunonegative presynaptic profiles. ***B4-B5***. Two examples of eGFP-Geph(S270A)-positive GC profiles in the EPL (*) making symmetric synapses labeled for gephyrin (arrows). ***B6***. Double-labeled GC profile, positive for GFP (*) and vGAT (#) forming a reciprocal synapse with a MC dendrite profile (arrowhead points to the glutamatergic postsynaptic density). Scale bars: 0.5 μm.

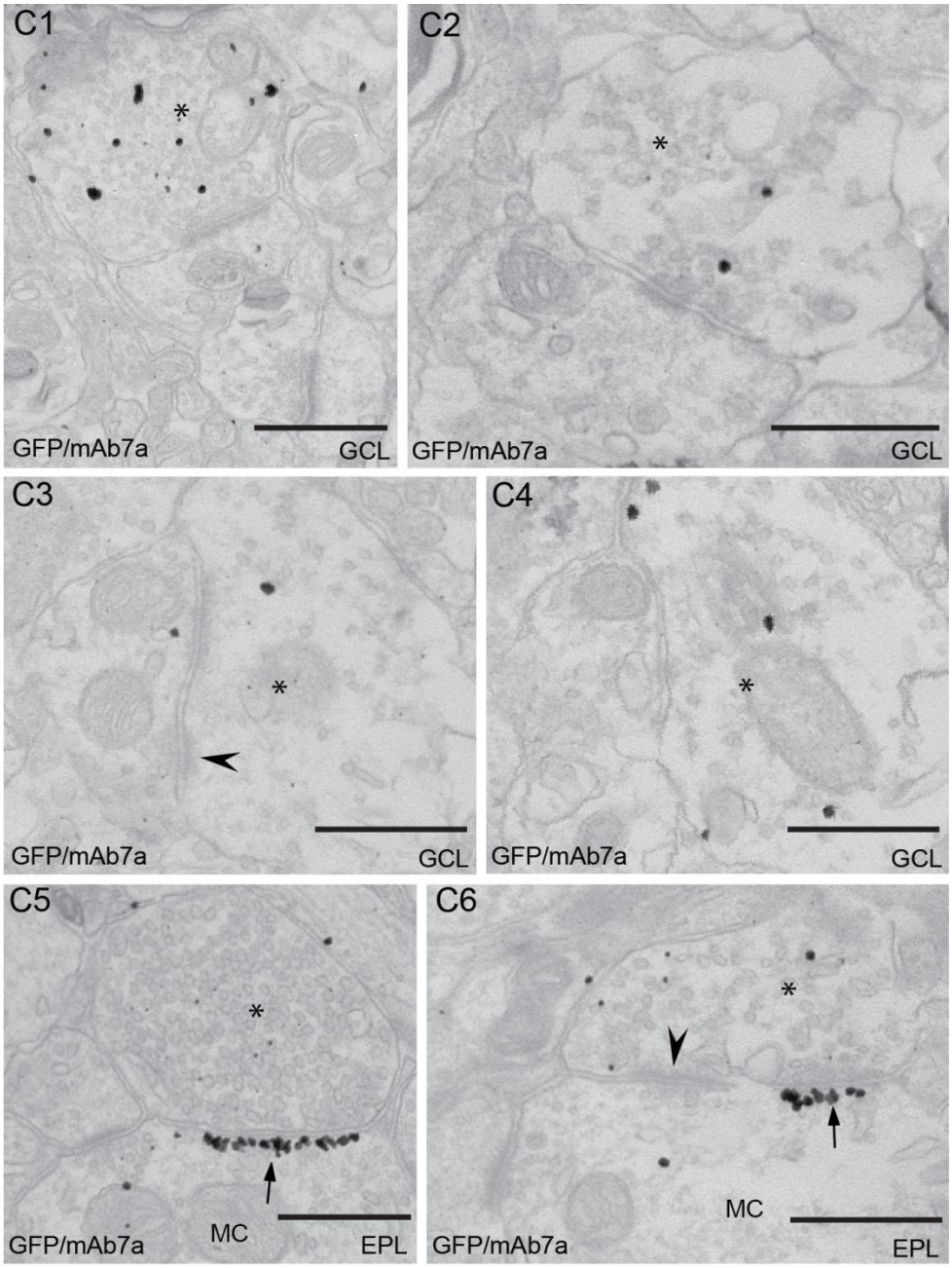


Figure 7 Double-labeling immunoelectron microscopy of adult-born GCs expressing eGFP-Geph(L2B) at 21 dpi. ***C1-C4***. Examples of eGFP-Geph(L2B)-positive profiles (*) in the GCL making (**C1**, **C2**) or receiving (**C3**, **C4**) synaptic contacts devoid of gephyrin labeling; in panels **C1** and **C2**, the postsynaptic density is unusually thick for being a GABAergic synapse; in **C3**, the GFP-positive profile receives a presumptive glutamatergic synapse (arrowhead). ***C5-C6***. eGFP-labeled profiles (*) in the EPL forming either a symmetric or a reciprocal synapse with strong gephyrin immunoreactivity; arrowhead points to the postsynaptic density of the glutamatergic synapse. Scale bars: 0.5 μm.

Discussion

The results demonstrate that the ability of neurons to form GABAergic synapses containing a postsynaptic gephyrin scaffold is crucial for their morphological differentiation, synaptic integration, and long-term survival in adult brain. Remarkably, over-expression of eGFP-Geph in adult-born GCs had almost no detectable effect, indicating that excess of gephyrin does not interfere with neuronal differentiation and GABAergic synapse formation. In contrast, over-expression of eGFP-Geph(S270A), which cannot be phosphorylated on Ser270, favored dendritic branching and the formation of transient GABAergic synapses on spines, containing α2-$GABA_A$Rs. This observation suggests that gephyrin phosphorylation at Ser270 (Tyagarajan et al., 2011b, Kuhse et al., 2012) is a mechanism that limits structural plasticity in both immature and mature GCs. Finally, over-expression of a gephyrin construct preventing its aggregation (eGFP-Geph(L2B)) – and, thereby, formation of postsynaptic scaffolds – largely precluded dendritic growth and branching, as well as formation of transient GABAergic synapses on spines. While this effect might be due to, at least in part, defective GABAergic transmission, we favor the view that it reflects the absence of signaling molecules anchored to the gephyrin scaffold, such as collybistin (Kins et al., 2000) and other gephyrin interactors that modulate the actin cytoskeleton (Maas et al., 2009) and thereby spine formation and dendrite branching. Since spines of adult-born GCs eventually the recipient of glutamatergic synapses in both the GCL and the EPL, a deficit in spine formation likely causes reduced excitatory transmission and its associated Ca^{2+} signaling, further having an impact on neuronal differentiation (Kelsch et al., 2012). This vicious circle eventually curtails long-term neuronal survival, as shown by our analysis at 14 and 90 dpi of GCs expression eGFP-Geph(L2B) and our modeling data (Fig. 3).

We observed in transfected GCs that over-expression of eGFP-Geph leads not only to formation of postsynaptic clusters, but also to the accumulation of gephyrin in spines that are pre/postsynaptic to mitral cell dendrites and postsynaptic to glutamatergic axon terminals. In addition, when amplified by immunofluorescence, diffuse eGFP-Geph was detectable throughout the cell, except in filopodia. These observations indicate either that gephyrin might have additional functions in neurons (including presynaptic structures and glutamatergic synapses) or that distinct mechanisms are required to ensure its selective clustering in GABAergic postsynaptic densities, while preventing its aggregation in other subcellular compartments. The numerous post-translational modifications of gephyrin, which include phosphorylation, acetylation, SUMOylation, S-Nitrosylation, and palmytoylation provide potential molecular

mechanisms underlying selective aggregation at GABAergic (and glycinergic) postsynaptic sites (Dejanovic et al., 2014, Tyagarajan and Fritschy, 2014). The failure of eGFP-Geph(L2B) to form postsynaptic clusters, which so far had been reported only *in vitro* (Lardi-Studler et al., 2007), was confirmed here, underscoring the importance of the surface-exposed loop encoded in this sequence. Remarkably, the L2B mutation disrupts a consensus site for binding to protein phosphatase 1, potentially implicating this site in the structural deficit preventing gephyrin clustering.

The need of immunofluorescence amplification in cells transduced with eGFP constructs precluded an electrophysiological analysis of single-transfected cells. Owing to this technical limitation, we were no able to verify whether the changes in structural plasticity seen upon expression of either eGFP-Geph(S270A) or the eGFP-Geph(L2B) were due to alterations in GABAergic synaptic transmission.

Significance of the gephyrin S270A point-mutation

The residue Ser270 is among the best characterized gephyrin residues that are phosphorylated *in vivo*. We and others have shown that its phosphorylation is mediated by GSK3β and cdk-5 (Tyagarajan et al., 2011b, Kuhse et al., 2012, Rui et al., 2013, Tyagarajan et al., 2013). Abolishing its phosphorylation by a Ser to Ala substitution or blocking the activity of GSK3β leads to the formation of supernumerary gephyrin clusters in cultured neurons, as well as to an increase in dendritic growth and branching, accompanied by enhanced GABAergic synaptic transmission. While the underlying mechanism is not clarified, previous evidence showed that GABAergic transmission is essential in the regulation of dendritic growth, in association with a higher stability of microtubules (Gascon et al., 2006). Furthermore, our results imply that gephyrin phosphorylation modulates its capacity to interact with (or activate) molecules involved in synaptogenesis and dendritic growth. Remarkably, however, there was no overall increase in spine density over time (Fig. 5), although GCs expressing eGFP-Geph(S270A) have a higher spine density than control cells in both the GCL and EPL, notably spines containing the α2-subunit. For this reason, we conclude that eGFP-Geph(S270A) favors the formation of transient, short-lived spines, as seen in immature cells. Therefore, *in vivo*, homeostatic mechanisms likely exist, which limit the formation of supernumerary spines in adult-born GCs. Likewise, quantification of α2-subunit clusters on dendritic shafts of GCs expressing eGFP-Geph(S270A) showed no increase over time, confirming a possible homeostatic regulation. Taken together, these findings indicate that eGFP-Geph(S270A) may have the ability to keep the cell in a state of

plasticity, which is normally observed at initial phases of their maturation (Whitman and Greer, 2007, Kelsch et al., 2009, Pallotto et al., 2012).

Consequences of preventing post-synaptic gephyrin clustering

In the Geph(L2B) mutant, 8 out of 10 residues (DIDGVRKI) forming a surface-exposed loop in the E-domain of rat gephyrin were replaced by their homologues from bacterial MoeA (KL..SNSW). As a consequence, gephyrin aggregation is severely impaired. Remarkably, replacing only 6 residues (IDGVRK) by their homologues (L..SNS) strongly increases the aggregation propensity of gephyrin, leading to formation of supernumerary postsynaptic clusters (Lardi-Studler et al., 2007). Neither mutation affects the enzymatic activity of gephyrin for Moco synthesis, suggesting that this surface-exposed loop, which is poorly conserved phylogenetically, is a key regulator of postsynaptic gephyrin function. While the L2B mutation is dominant-negative, we observed the formation of a few gephyrin clusters labeled by mAb7a as well some α2-subunit clusters, as previously reported *in vitro*. Accordingly, over-expression of eGFP-Geph(L2B) might not fully abolish synaptic GABAergic function. Nevertheless, the consequences on the structural development and survival of adult-born GCs were stronger than after abolition of α2-$GABA_A$Rs, indicating that interfering with postsynaptic gephyrin clustering has pronounced detrimental effects during neuronal development. The long-term effects, including the limited life-span of GCs over-expressing eGFP-Geph(L2B), are likely related to the impairment of dendritic growth and spine formation, thereby affecting synaptic integration and appropriate down-stream signaling. It is noteworthy that, ultrastructurally, dendrite and spine profiles appeared unaltered, and reciprocal synapses could readily be detected in the EPL at 21 dpi. Therefore, the eGFP-Geph(L2B) mutation severely limits the ability of cells to form transient GABAergic synapses on spines, opposite to the phenotype of eGFP-Geph(S270A). Consequently, it is this "plasticity deficit" rather than a "structural deficit" that negatively affects the formation of spines and dendritic branching.

Signaling at the gephyrin scaffold

There are at least two possible mechanisms, not mutually exclusive, explaining the opposite phenotype of GCs expressing eGFP-Geph(S270A) and eGFP-Geph(L2B). First, owing to the fact that gephyrin interacts with numerous signaling molecules (see Introduction), failure to form a postsynaptic scaffold might affects down-stream signaling contributing to remodeling of the cytoskeleton and spine formation. In particular, the control of the small GTPases Cdc42 and TC-10 activity by gephyrin-collybistin interaction (Papadopoulos et al., 2007, Mayer et al., 2013) is

an obvious candidate. Second, reduced spinogenesis limits the formation of afferent synapses onto adult-born GCs, both GABAergic and glutamatergic. The former might generate depolarizing signals, notably in immature cells, leading to activation of NMDA-receptors (Kelsch et al., 2012) and opening of voltage-dependent Ca^{2+} signals. These contribute to activation of protein kinases and phosphatases, including CaMKII and calcineurin, which play a key role in the activation of down-stream Ca^{2+} dependent signaling pathways and transcription factors, in particular CREB, which is necessary for maturation and survival of newborn neurons (Obrietan et al., 2002, Jagasia et al., 2009) and their morphological differentiation (Giachino et al., 2005, Herold et al., 2011). Additional conceivable mechanisms include Ca^{2+}-dependent activation of calpain, a neuron-specific protease that regulates the function of a vast array of synaptic and structural proteins and is known to modulate gephyrin postsynaptic clustering (Tyagarajan et al., 2011b). Therefore, the focus of future work will be to elucidate how gephyrin scaffolds modulate spine formation, in particular at immature stages when newborn cells still receive only limited glutamatergic input.

In conclusion, the present work uncovers a key role of gephyrin as a scaffolding molecule in GABAergic postsynaptic densities for modulating structural plasticity in developing neurons, thereby expanding the repertoire through which the GABAergic system regulates neuronal development and maturation.

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STUDY II: A PARTIAL, BUT NOT A FULL, INACTIVATION OF α5 SUBUNIT-CONTAINING $GABA_A$ RECEPTORS IN MICE AFFECTS THE DEVELOPMENT OF ADULT-BORN NEURON IN THE HIPPOCAMPUS

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Fabia Vogt performed experiments with wild-type, α5-KO and $\alpha5^{fl/fl}$ mice and analyzed their data. I performed experiments with α5-het mice and analyzed the data, and wrote the paper.

Abstract

Alteration in hippocampal activity due to changes in $GABA_A$ receptors ($GABA_AR$) mediating tonic inhibition influences different hippocampal functions. *Gabra5*-null mice and mutant mice carrying a histidine-to-arginine point mutation at residue 105 (α5H105R) exhibit signs of hippocampal dysfunction, but are capable of improved performance in several learning and memory tasks. Accordingly, alleviating abnormal GABAergic tonic inhibition in the hippocampal formation by selective α5-$GABA_AR$ modulators has been proposed as a therapeutic approach for several intellectual deficit disorders. Adult neurogenesis in the dentate gyrus is an important facet of hippocampal plasticity, known to be regulated by tonic GABAergic transmission, as shown by deficits in proliferation, migration, and dendritic development of newborn neurons in *Gabra4*-null mice. Here, we investigated the contribution of α5-$GABA_ARs$ to the regulation of adult-born granule cell development, using birth-dating and labeling of precursor cells by transduction with retroviral vectors expressing eGFP. Global α5-$GABA_AR$ knockout mice showed no alterations in migration and morphological development of eGFP-positive granule cells. However, an up-regulation of α1, α3, and α4 subunit-immunoreactivity was observed in distinct regions of the hippocampal formation. In contrast, a partial reduction of α5-$GABA_ARs$ in α5-heterozygous mice, as well as single-cell deletion of *Gabra5* selectively in newborn granule cells from α5-floxed mice, causes severe alterations of migration and dendrite development of adult-born granule cells. These results reinforce the notion that subtle alterations in neuronal and network excitability due to an imbalance of α5-$GABA_AR$-mediated transmission has major consequences for neuronal development and plasticity; and call for caution about the chronic use of negative allosteric modulators acting at these receptors.

Introduction

$GABA_A$ receptors ($GABA_ARs$) containing the α5 subunit are highly expressed during brain development and have a restricted distribution in adult CNS (reviewed in (Fritschy and Panzanelli, 2014). Representing <10% of total $GABA_ARs$, they are most abundant in the hippocampus, amygdala, olfactory bulb, brainstem, and spinal cord (Fritschy and Mohler, 1995, Fritschy et al., 1998, Serwanski et al., 2006). The majority of α5-$GABA_ARs$ are located extrasynaptically, mediating tonic inhibition (Xing et al., 2004, Prenosil et al., 2006, Glykys et al., 2008, Belelli et al., 2009). However, in the hippocampus, they also contribute to several forms of slow synaptic inhibition, suggesting specialized functions in the control of neuronal excitability (Glykys and Mody, 2006, Zarnowska et al., 2009, Vargas-Caballero et al., 2010).

The analysis of mutant mice carrying a histidine-to-arginine point mutation at residue 105 (α5H105R), which have a ~30% decrease in α5-$GABA_ARs$ in the CNS, revealed that these mice exhibit several signs of hippocampal dysfunction, including locomotor hyperactivity, reduced pre-pulse inhibition, and impaired memory for the location of objects (Hauser et al., 2005, Prut et al., 2010). Paradoxically, however, these mice also exhibited improved performance in several learning and memory tasks, in particular trace fear conditioning, and are more resistant to extinction of conditioned fear responses (Crestani et al., 2002, Yee et al., 2004). The latter findings have been replicated in *Gabra5*-null mice (Collinson et al., 2002). Accordingly, negative allosteric modulators acting specifically at α5-$GABA_ARs$ have cognitive enhancing properties (Dawson et al., 2006) and allow to revert memory deficits observed in several experimental pathological conditions (Lieberwirth et al., 2012). Altered α5-$GABA_AR$ functions have also been reported in models of schizophrenia (Gill and Grace, 2014) and cognitive disability, including Fragile X syndrome and Down syndrome, along with symptomatic improvement following treatment with selective allosteric modulators (reviewed in (Rudolph and Mohler, 2014)). Furthermore, α5-$GABA_ARs$ selectively contribute to the development of tolerance against the sedative (motor-impairing) action of chronic diazepam administration (van Rijnsoever et al., 2004), a mechanism accompanied by a reduced expression of α5-$GABA_ARs$ selectively in the dentate gyrus (DG). Collectively, these observations point to tonic (and slow) inhibitory neurotransmission mediated by α5-$GABA_ARs$ as a key regulator of hippocampal function for learning and memory.

Tonic $GABA_AR$-mediated inhibition is also well known to modulate adult neurogenesis in rodent CNS, notably by regulating stem and neural precursor cell proliferation (Liu et al., 2005, Song et

al., 2012b), migration of newborn neurons in the granule cell layer (GCL), and their dendritic maturation (Duveau et al., 2011). However, many of these effects are mediated by α4-$GABA_A$Rs whereas the contribution of α5-$GABA_A$Rs has not been established. Therefore, the aim of the present study was to determine whether reduced function of α5-$GABA_A$Rs upon targeted deletion of *Gabra5* affects specific steps of adult neurogenesis in the DG. To this end, we investigated the development of adult-born granule cells (GCs) birth-dated and labeled by transduction with a retroviral vector encoding eGFP in global α5-$GABA_A$R knockout (α5-KO) and heterozygous (α5-het) mice, compared to wild-type control. Furthermore, we investigated the consequences of a cell-autonomous *Gabra5* deletion using the Cre/lox system to determine the selective role of α5-$GABA_A$Rs for the maturation of newborn GCs.

Materials and Methods

Animals

All experiments were performed in accordance with the European Community Council Directives of November 24, 1986 (86/609/EEC) and approved by the cantonal veterinary office of Zurich. In the present study, we used 8 to 12 week-old male C57BL7/6J (wild-type; wt), α5-knockout (α5-KO), heterozygous (α5-het), and α5-floxed ($\alpha5^{fl/fl}$) mice, which were bred in the dedicated animal facility of the Institute of Pharmacology and Toxicology of the University of Zurich. $\alpha5^{fl/fl}$ and α5-KO mice were generated as described (Engin et al., submitted). Both sexes were used for the present study. Genotyping was confirmed by PCR analysis from ear biopsies.

Retroviral vectors

Retroviruses encoding the green-fluorescent protein (GFP), monomeric red fluorescent protein (mRFP), or Cre-GFP constructs were produced by transfecting HEK 293T cells with three separate plasmids containing the capsid (CMV-vsvg), viral proteins (CMV-gag/pol) and transgene (CAG-eGFP, CAG-mRFP and CAG-Cre-eGFP) under the control of the CAG promoter (including a cytomegalovirus (CMS) enhancer and chicken β-actin promoter). The supernatant containing the virus was concentrated by ultracentrifugation and diluted in PBS. The preparations used had a titer of at least 10^8 cfu/mL, as determined by serial titration upon HEK293 cell transfection.

Stereotaxic injections

Adult mice were anesthetized by inhalation with 2.5-3% isoflurane (Baxter) in oxygen and placed on the stereotaxic frame (David Kopf Instruments). The mice received a bilateral injection of retroviruses encoding eGFP (1 μL) or a 1:1 mixture of retroviruses encoding Cre-eGFP and mRFP (1.5 μL) into the dorsal hippocampus (anteroposterior (AP) = -2 mm, mediolateral (ML) = ±1.5 mm, dorsoventral (DV) = -2.3 mm, with Bregma as reference), using a nanoliter injector Nanoject II (Drummond Scientific). After the surgery, the animals received an i.p. injection of 1 mg/kg buprenorphine (Temgesic, Essex Chemicals, Lucerne, Switzerland) and were recovered from anesthesia on a warm pad, before returned to their home-cage.

Tissue preparation for immunohistochemistry

Mice were deeply anesthetized with pentobarbital (Nembutal®; 50 mg/kg, i.p.) and perfused transcardially with 15-20 mL ice-cold, oxygenated artificial CSF (ACSF), pH 7.4, as described

(Notter et al., 2014). After perfusion mice were decapitated and brains were immediately removed on ice and fixed by immersion in 4% paraformaldehyde (dissolved in 0.15 M sodium phosphate buffer, pH 7.4) for 90 min. After cryoprotection overnight in 30% sucrose, 50-µm-thick coronal sections containing the hippocampus from frozen brain were cut with a sliding microtome and collected in phosphate-buffered saline (PBS).

Immunofluorescence staining

Single or double or triple immunofluorescence staining was performed by incubating sections with primary antibodies against GFP (and where applicable, mRFP) (Table 1) diluted in PBS (pH 7.4) containing 2% normal goat serum and 0.2% Triton X-100 for 48-72 h at 4°C.

Sections were then washed in 3x PBS for 10 min and incubated at room temperature in secondary antibodies together with DAPI. Secondary antibodies conjugated to Alexa 488 (Invitrogen) or Cy3 (Jackson ImmunoResearch) were raised in goat. Afterwards, sections were rinsed again 3 times in PBS and mounted on gelatin-coated slides, before coverslipped with a fluorescence mounting medium (Dako).

Table 1: List of primary antibodies

Target protein	Species	Dilution	Source; catalog	Method
GFP	chicken	1:2000	Aves Laboratories; GFP-1020	IF
mRFP	rat	1:2000	Chromo Tek; RFP 5F8	IF
$GABA_AR$ α1-subunit	Guinea pig	1:30'000	Self-made; Fritschy and Mohler, 1995	Peroxidase
$GABA_AR$ α2-subunit	Guinea pig	1:2000	Self-made; Fritschy and Mohler, 1995	Peroxidase
$GABA_AR$ α3-subunit	Guinea pig	1:12'000	Self-made; Fritschy and Mohler, 1995	Peroxidase
$GABA_AR$ α4-subunit	rabbit	1:2000	PhosphoSolutions, Aurora	Peroxidase

Immunoperoxidase staining

Immunoperoxidase staining was performed using diaminobenzidine as a chromophore by incubating brain sections with the primary antibody (Table 1) diluted in Tris-Triton (pH 7.4) containing 2% normal goat serum and 0.2% Triton X-100 over night at 4°C. Sections were then washed in 3x Tris-Triton pH 7.4 for 10 min and incubated for 30 min at room temperature in

secondary biotinylated antibody (1:300, Jackson ImmunoResearch). Afterwards sections were again washed in Tris-Triton pH 7.4 for 3 times, followed by incubation in an ABC complex solution for 30 min (Vectastain Elite kit; Vector Laboratories) and reaction with diaminobenzidine tetrahydrochloride (Sigma-Aldrich) for 5-15 min in Tris buffer, pH 7.7. Finally, sections were washed thoroughly, mounted onto gelatin-coated slides, air-dried overnight, dehydrated, and coverslipped with Eukitt (Erne Chemie).

Image acquisition and analysis

Densitometry analysis

Immunoperoxidase stained sections were examined by bright-field microscopy (Carl Zeiss AG, Jena, Germany) using 20x objective or 40x oil immersion objective. Images were acquired with an 8 bit digital color camera controlled by AxioVision 4.5 (Carl Zeiss, AG, Jena, Germany).

Densitometry analysis of different $GABA_AR$ α-subunits was performed in wild-type, α5-het and α5-KO littermates (3-4 mice per group). Images were digitized on a Northern light Model B95 light box (Imaging Research Inc., Brock University, St.Catharines, ON, Canada) using a CoolSnap digital camera (Photometrics, Tuscon, AZ, USA) with a Micro-Nikkor 55mm + 12mm objective (Nikon Corporation). Images were acquired from three regions of interest: CA1, CA3 and the DG of the hippocampus, as well as laterodorsal thalamic nucleus. To reduce the alterations in staining intensity, the ROD value of the corpus callosum was taken as background and subtracted from the regions of interest from the same section.

Dendritic development

To visualize morphology of newborn neurons, a laser-scanning microscopy (LSM700, Carl Zeiss) with a 25x or 40x (NA, 1.4) oil immersion objective was used. Sequential acquisition of separate wavelength channels was performed to avoid fluorescence crosstalk.

The total length and number of branches of the dendritic tree of newborn GCs was quantified by Sholl analysis in all three genotypes. The entire thickness of the cell was acquired, taking z-stacks with the LSM700 spaced by 0.7 μm. Dendritic morphometry was analyzed with the NeuronJ plug-in from the software NIH ImageJ. Sholl analysis was performed with concentric circles, spaced at 10 μm intervals, centered on the cell body. The numbers of intersections were calculated using a Sholl Analysis plugin (Anirvan Ghosh Laboratory, University of California, San Diego, La Jolla, CA). For statistical comparison, the area under the curve (AUC) of the

resulting function was calculated. Quantifications were performed on 13-20 cells from 3-6 mice for each animal at each time-point.

Migration distance

The distance of migration of newborn neurons was measured from images of the GCL containing either eGFP-positive (wild-type, α5-het and α5-KO mice) or eGFP/mRFP-positive cells ($\alpha5^{fl/fl}$ mice), taken by the LSM700. Using DAPI to counterstain nuclei in the GCL, the distance was measured perpendicularly to a virtual line through the base of the GCL. For each animal (3-6 mice per group) and each time-point, 20-60 cells were analyzed.

Spine density

In order to calculate spine density at least 5 images of eGFP-positive dendritic segments of wt, KO and heterozygous animals after 28 and 42 dpi (n = 3-4) were acquired using Z-stacks with an interval of 0.7 µm to capture the whole segment. Pictures were obtained randomly using a 40x oil immersion objective with digital zoom factor 2.0 (pixel size, 60 nm). No distinction was made between spine and filopodia.

Spines on the segment were counted using ImageJ applying the plugin CellCounter. For each segment the length was measured and number of spines/µm was then calculated.

Statistical analyses

Data are presented as mean± SEM. Statistical analyses were made using one-way ANOVA followed by an unpaired t-test and two-way ANOVA, followed where appropriate by Tukey *post-hoc* tests (Prism software; GraphPad, version 6). Cumulative probability distributions, used for migration distance, were compared using the Kolmogorov-Smirnov test. Statistical significance was set at $P<0.05$.

Results

Among the various steps of adult neurogenesis in the DG, we investigated here the effects of full, partial, or cell-specific inactivation of *Gabra5* on the migration of newborn GCs into the GCL, their morphological differentiation, and the formation of dendritic spines. For studying the effects of full or partial gene inactivation, we stereotaxically injected a retroviral vector encoding eGFP into the dorsal hippocampus of adult wild-type, α5-KO and α5-het mice and analyzed them morphologically at 7, 14, 28 and 42 days post-injection (dpi). Previous work showed that upon abolishing tonic $GABA_AR$-mediated inhibition in GCs by targeted deletion of *Gabra4*, migration and dendrite formation were impaired (Duveau et al., 2011).

Positioning of newborn neurons in the GCL

Post-mitotic GC precursors migrate from the SGZ to their final position in the GCL while undergoing morphological and functional differentiation (Kempermann et al., 2004). To quantify migration distance at each of the three selected time-points, coronal sections through the dorsal hippocampus were stained against GFP and DAPI, allowing the visualization of newborn GCs and their location in the GCL. Their migration distance away from the SGZ was determined as the shortest (orthogonal) distance between the cell center and the SGZ - GCL boundary. At 7 dpi, the soma of eGFP-positive GCs from wild-type and α5-KO mice was located either in the SGZ or a few μm away in the GCL, whereas about 30% labeled cells in α5-het mice had migrated in the opposite direction (towards the hilus), while the others remained close to the SGZ (Fig. 1A,B). At 14 and 28 dpi, GCs from wild-type and α5-KO mice had migrated further into the GCL, typically in the inner third, whereas at 42 dpi the majority was seen the middle third of the GCL (Fig. 1A',C-D). No difference between wild-type and α5-KO mice was observed at any time-point. In contrast, eGFP-positive GCs from α5-het mice migrated less deeply into the GCL, and those that went towards the hilus did not reverse their course. Pair-wise comparison of genotypes using Kolmogorov-Smirnov analysis revealed a significant difference between α5-het and either wild-type or α5-KO mice at each time-point analyzed (Fig. 1B-E).

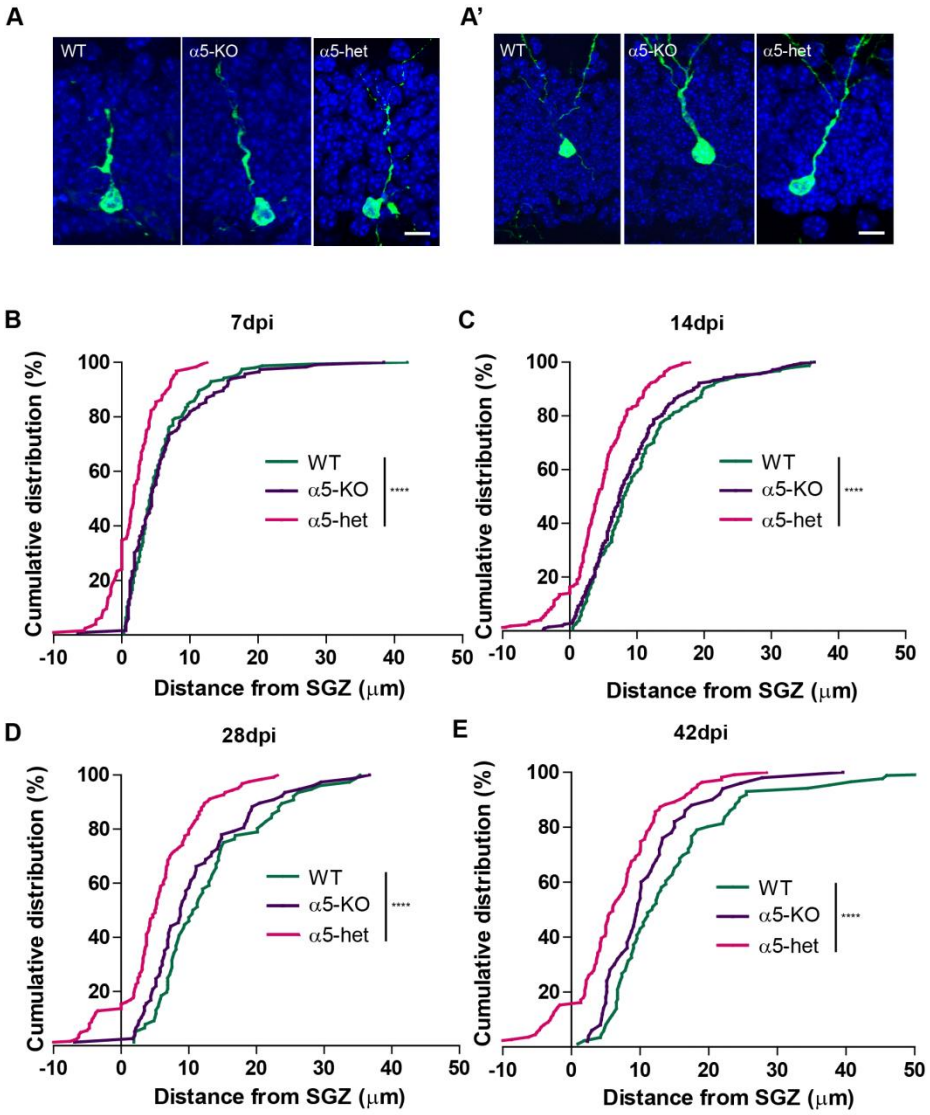


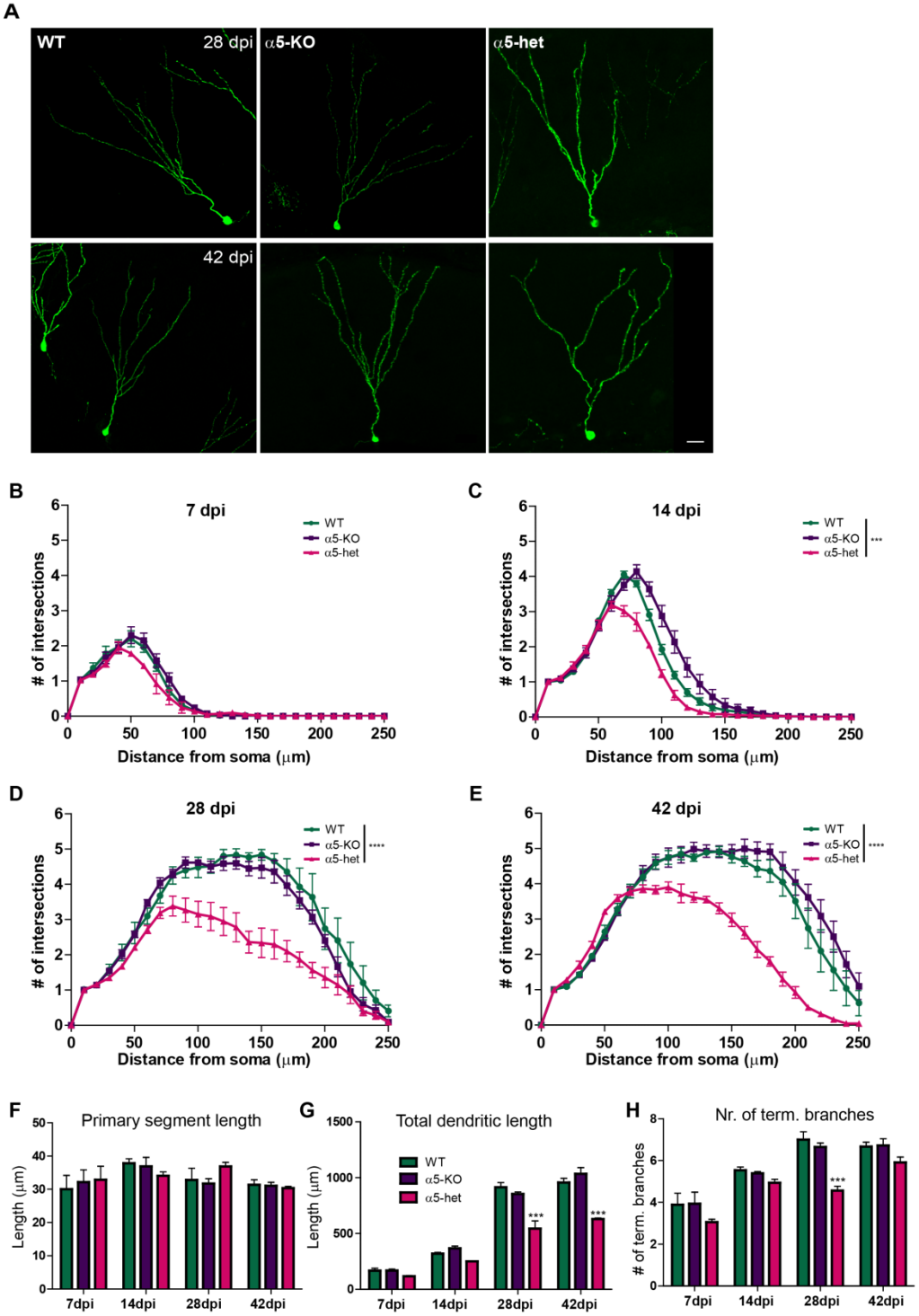
Figure 1. Migration distance of newborn GCs in wild-type, α5-KO and α5-het animals. ***A,A'.*** Representative images illustrating the localization of eGFP-positive soma of newborn GCs at 7 dpi and 42 dpi in the GCL (blue) of wild-type and mutant mice. ***B-E.*** Cumulative distribution analysis of the migration distance in the GCL of eGFP-positive GCs at 7 dpi (B), 14 dpi (C), 28 dpi (D) and 42 dpi (F). Between wild-type and α5-KO newborn GCs, no significance difference was observed, whereas newborn GCs of α5-het mice migrate significantly less deeply into the GCL at all time-points analyzed; a small fraction even migrates in the opposite direction (negative values) towards the hilus. Note that the fraction of these cells remains constant over time, indicating that they survive for at least 42 days. For statistical comparison, Kolmogorov-Smirnov test was used for pair-wise comparisons of the distribution curves (****P<0.0001 compared to wild-type eGFP-positive GCs). Scale bar: 10 μm.

Dendritic development

To determine whether full or partial ablation of the α5-$GABA_AR$ impairs dendrite growth and branching, as seen in *Gabra4*-null mice (Duveau et al., 2011), we quantified major morphometric parameters (dendrite arbor complexity, total dendritic length, primary segment length, number of terminal branches) in eGFP-positive newborn GCs. At 7 dpi, all cells had a distinct apical dendrite extending into the GCL, as see in three genotypes, but this time-point was not quantified. At 14 dpi, the spine-less dendritic tree extended into the inner molecular layer, whereas at 28 dpi, distal branches extended almost up to the outer border of the DG, and spine formation could be observed (Fig. 2A). Finally, at 42 dpi, eGFP-positive GCs exhibited a well-developed, mature-like dendritic tree (Fig. 2A). Quantification of the dendritic arbor complexity by Sholl analysis revealed no significant differences between wild-type and α5-KO mice at any time-point, although a progressive increase in dendritic arborization was observed over time. In contrast, α5-het mice exhibited a striking reduction of dendritic complexity compared to either control of α5-KO mice (Fig. 2B-E). At each time-point, quantification of the area-under-the-curve by one-way ANOVA confirmed these observations by revealing a significant effect of genotype: 14 dpi: $F_{(2,11)} = 13.83$, $P = 0.001$; 28 dpi: $F_{(2,9)} = 18.14$, $P = 0.0007$; 42 dpi: $F_{(2,9)} = 16.05$, $P = 0.0011$) (Fig. 2B-E).

While the length of the primary segment (from the soma to the first bifurcation) remained constant all time points analyzed and between genotypes (Fig. 2F; two-way ANOVA), quantification of total dendritic length revealed a significant effect of time ($F_{(3.39)} = 385.8$, $P<0.0001$) and genotype ($F_{(2.39)} = 63.25$, $P<0.0001$), as well as a significant interaction ($F_{(6.39)} = 10.31$, $P<0.0001$). *Post-hoc* analysis revealed that eGFP-positive GCs from α5-het mice exhibited a significant reduction in total dendritic length compared to wild-type control at 28 ($P<0.001$) and 42 dpi ($P<0.001$); however, no differences could be detected between wild-type and α5-KO mice (Fig. 2G). The significant time x genotype interaction likely indicates that dendrite growth in GCs from α5-het mice is retarded compared to control and α5-KO mice. Additionally, quantification of number of terminal branches revealed a significant effect of time ($F_{(3,41)} = 36.44$; $P<0.0001$) and genotype ($F_{(2,41)} = 12.77$; $P<0.0001$), but no interaction, indicating a similar time-dependent evolution of branching. However, *post-hoc* analysis indicated that, whereas newborn neurons form wild-type and α5-KO mice did not differ from each other, newborn GCs from α5-het mice had a reduced number of terminal branches at 28 dpi ($P<0.001$) (Fig. 2H).

The results so far unexpectedly show that constitutive *Gabra5* inactivation caused no effect on migration and dendritic development in adult-born GCs, whereas a partial deletion had strong and enduring effects on these processes that are essential for their proper functional integration.



(Figure 2: Legend on next page)

Spine density

Extrasynaptic α5-$GABA_A$Rs have been postulated to be well positioned for modulating the spread of excitation from glutamatergic synapses formed on spines. It was, therefore, of major interest to determine whether ablation of α5-$GABA_A$Rs would affect spine formation. Quantification of spine density (number of spines per μm dendrite) at 28 dpi in intermediate and distal dendritic segments in the ML of eGFP-positive neurons in wild-type (1.37±0.07 spines/μm), α5-KO (1.39±0.03 spines/μm) and α5-het (1.41±0.05 spines/μm) mice revealed no difference between genotypes. Likewise, at 42 dpi spine density was uniform across genotypes (wild-type: 1.43±0.01 spines/μm; α5-KO: 1.48±0.1 spines/μm; α5-het: 1.57±0.08 spines/μm) (Fig. 3A-B). Further, two-way ANOVA analysis revealed no significant effect of time ($F_{(1,11)}$ = 4.793; P = 0.0510), indicating that maximal spine density is achieved already at 28 dpi. These data are in line with our previous results in *Gabra2*- and *Gabra4*-null mice (Duveau et al., 2011) and confirm that spine formation in GCs might be governed by extrinsic influences from the perforant path (Frotscher et al., 2000). Further, similarly to CA1 pyramidal cells (Bonin et al., 2007), they imply that adult-born GCs from α5-KO and α5-het mice might be hyper-excitable.

Figure 2 Dendritic development of eGFP-positive newborn neurons in wild-type, α5-KO and α5-het mice.
A. Representative images of dendritic arborization at 28 and 42 dpi of eGFP-positive newborn GCs, illustrating the reduced complexity in α5-het mice. ***B-E.*** Quantification of dendritic arborization by Sholl analysis at 7, 14, 28 and 42 dpi. The number of intersections between eGFP-positive dendrite segments and virtual concentric lines centered on the cell body and spaced by 10 μm is depicted (mean SEM; N=3-6 mice/group). For statistical comparison between groups, the area-under-the-curve was compared by one-way ANOVA at each time-point (P<0.05). No difference in dendritic complexity was observed between wild-type and α5-KO mice. Newborn neurons in α5-het mice had normal growth of dendrites up to 7 dpi, but became significantly different at 14 (P =0.001), 28 (P =0.0007) and 42 dpi (P =0.0011). ***F-H.*** Quantification of proximal dendritic length, total dendritic length and number of terminal branches, represented by bar-graphs (mean SEM; N=3-6 mice per group). ***P<0.001, compared to eGFP-positive wild-type cells; Tukey *post-hoc* tests). No significant difference was observed between wild-type and α5-KO mice for all morphological parameters. Newborn neurons of α5-het mice showed a significant decrease in total dendritic length at 28 and 42 dpi, and a decrease in terminal branches at 28 dpi. Scale bar: 20 μm.

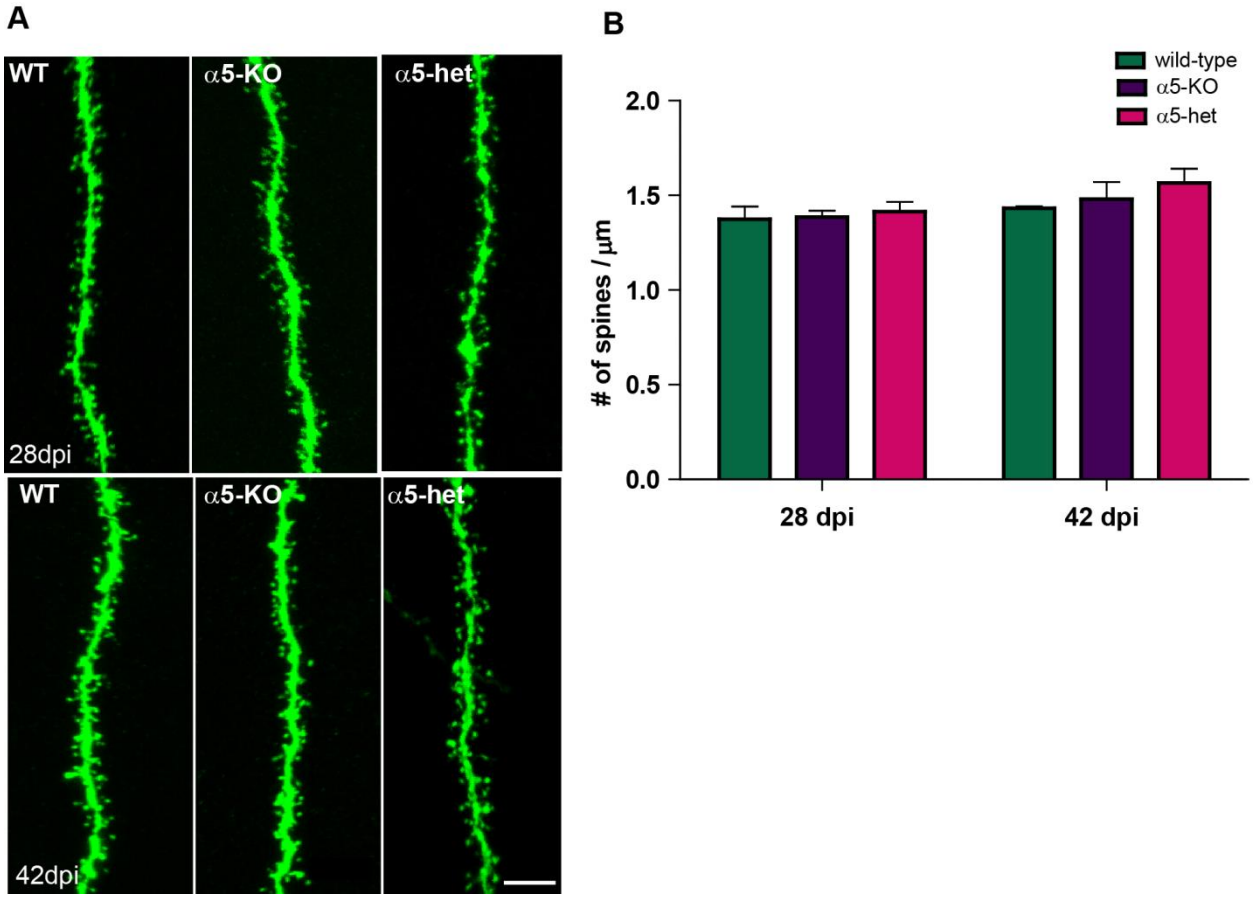


Figure 3 Spine density. ***A.*** Representative images of spines on randomly chosen distal dendritic segments of wild-type, α5-KO and α5-het mice at 28 and 42 dpi. ***B.*** Quantification of spine density (mean±SEM; N=3-4 mice per group) indicated as number of spines/µm. No significant difference was observed between the three genotypes at either time-point (one-way ANOVA). Scale bar: 5 µm.

Compensatory changes in $GABA_AR$ α subunit expression in the hippocampal formation

Considering the lack of phenotype seen so far in adult-born GCs from α5-KO mice, we wondered whether α5-$GABA_ARs$ might be replaced by over-expression of another α subunit variant. Although immunohistochemistry is not a quantitative method, it allows differentiating the relative abundance of a given protein on a regional or even cellular basis. Therefore, expecting that a compensatory increase might be most prominent in regions enriched in α5-$GABA_ARs$, we compared the regional distribution of the α1-α4 subunits in brain sections from naïve wild-type, α5-KO, and α5-het littermates, as detected by immunoperoxidase staining (Fig. 4A). For each subunit, we measured the relative staining intensity in the CA1 and CA3 region, as well as for the DG. No change was observed across genotypes for the α2 subunit immunoreactivity (Fig. 4C).

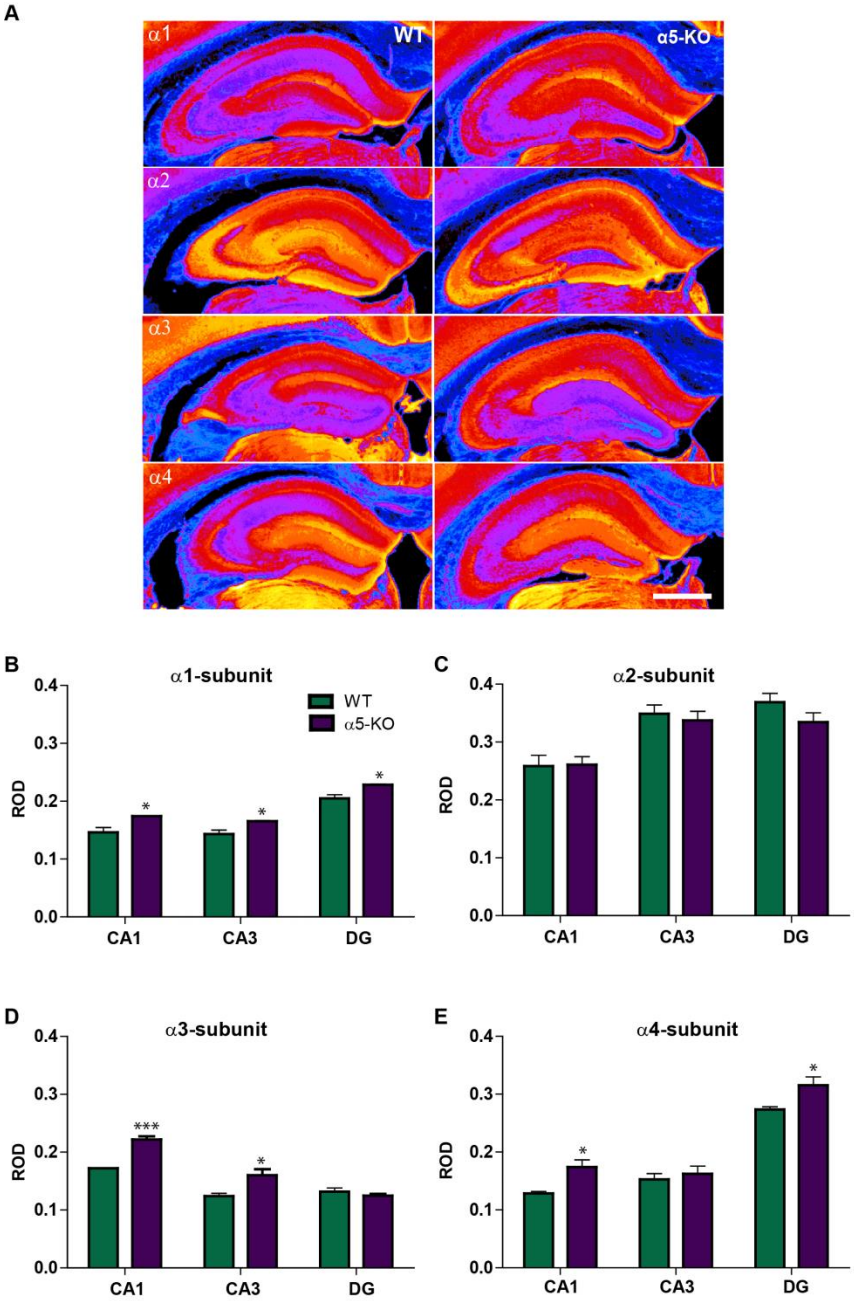


Figure 4 Expression levels of different α subunit containing-GABA$_A$Rs.
A. Representative digital images depicting in false-color the relative intensity of α1, α2, α3, and α4 subunit-immunoreactivity in the hippocampal formation of wild-type and α5-KO mice. For each antibody, the strongest signals are depicted in white, followed by yellow, orange, pink, cyan, and blue. ***B-D.*** Quantitative representation of ROD values of the immunoreactivity of the different α subunit in the CA1 region, CA3 and DG of the hippocampus. Compared to wild-type mice, α5-KO mice exhibited elevated α1 subunit immunoreactivity in CA1, CA3 and DG (B); α3 subunit staining was increased in CA1 and CA3 (D); and the α4 subunit-immunoreactivity was elevated in CA1 and DG (E); whereas the α2 subunit staining remained unchanged between the two genotypes (C). For statistical comparison, two-way ANOVA with Bonferroni *post-hoc* tests was used (*P<0.05; ***P<0.001). Scale bar: 0.2 mm.

However, staining intensity of the α1 subunit was significantly elevated in all three regions in α5-KO mice (two-way ANOVA; genotype: $F_{(1,18)} = 32.42$, $P<0.0001$; region: $F_{(2.18)} = 84.54$, $P<0.0001$) (Fig. 4B). The α3-subunit staining was increased in the CA1 ($P =0.001$) and CA3 ($P = 0.036$) region, but not in the DG of α5-KO mice (two-way ANOVA; genotype: $F_{(1,15)} = 25.23$, $P = 0.0002$; region: $F_{(2,15)} = 64.17$, $P<0.0001$) (Fig. 4D). Finally, the α4-subunit staining intensity was significantly up-regulated in the CA1 ($P = 0.012$) and in the DG ($P = 0.032$) but not in the CA3 region of α5-KO mice (two-way ANOVA; genotype: $F_{(1,18)} = 14.14$, $P = 0.0014$; region: $F_{(2,18)} = 116.5$, $P<0.0001$) (Fig. 4E). As a control, each of these subunits was also measured in the laterodorsal nucleus of the thalamus, where the α5 subunit is not expressed in adult brain. No significant change was observed (not shown). Therefore, these observations suggest that constitutive, but not partial, *Gabra5* deletion induces compensatory changes in the expression profile of other $GABA_A$Rs. It is not known, however, whether these changes directly affect $GABA_A$R expressed by adult-born GCs (at various stages of maturation), or only their local "environment".

Cell-autonomous effects following single-cell *Gabra5* gene deletion

To address this issue, we investigated the effects of single-cell *Gabra5* deletion, induced by Cre-recombinase-mediated excision of the exons of *Gabra5* flanked by LoxP sites, as described (Engin et al., submitted). We used a mixture of two retroviruses encoding either Cre-eGFP or mRFP for injection into the dorsal hippocampus of mice carrying floxed *Gabra5* alleles ($\alpha5^{fl/fl}$). Immunohistochemistry of brain sections against GFP and mRFP was performed at 14, 28 and 42 dpi. With this strategy, we expected to see in the same animal three populations of labeled newborn GCs, either single- or double-transfected. As Cre recombinase is targeted to the nucleus, mRFP in double-transfected cells allowed studying the cell morphology and quantifying dendrite arborization.

As above, we quantified migration distance and dendritic complexity of mRFP-positive ($\alpha5^{fl/fl}$) and double-transfected GCs. In contrast to the results seen in α5-KO mice, mutant cells in $\alpha5^{fl/fl}$ mice exhibited a significant reduction in the distance of migration (61.2%; $P<0.0001$; Kolmogorov Smirnov) compared to Cre-negative cells (Fig. 5A). In particular, around 10% of mutant GCs migrated towards the hilus, as seen in α5-het mice. At 28 dpi, mutant cells exhibited a 38.7% ($P<0.0001$), and at 42 dpi, a 24.6% reduction in migration distance ($P<0.0001$), compared to mRFP-positve cells (Fig. 5B,C).

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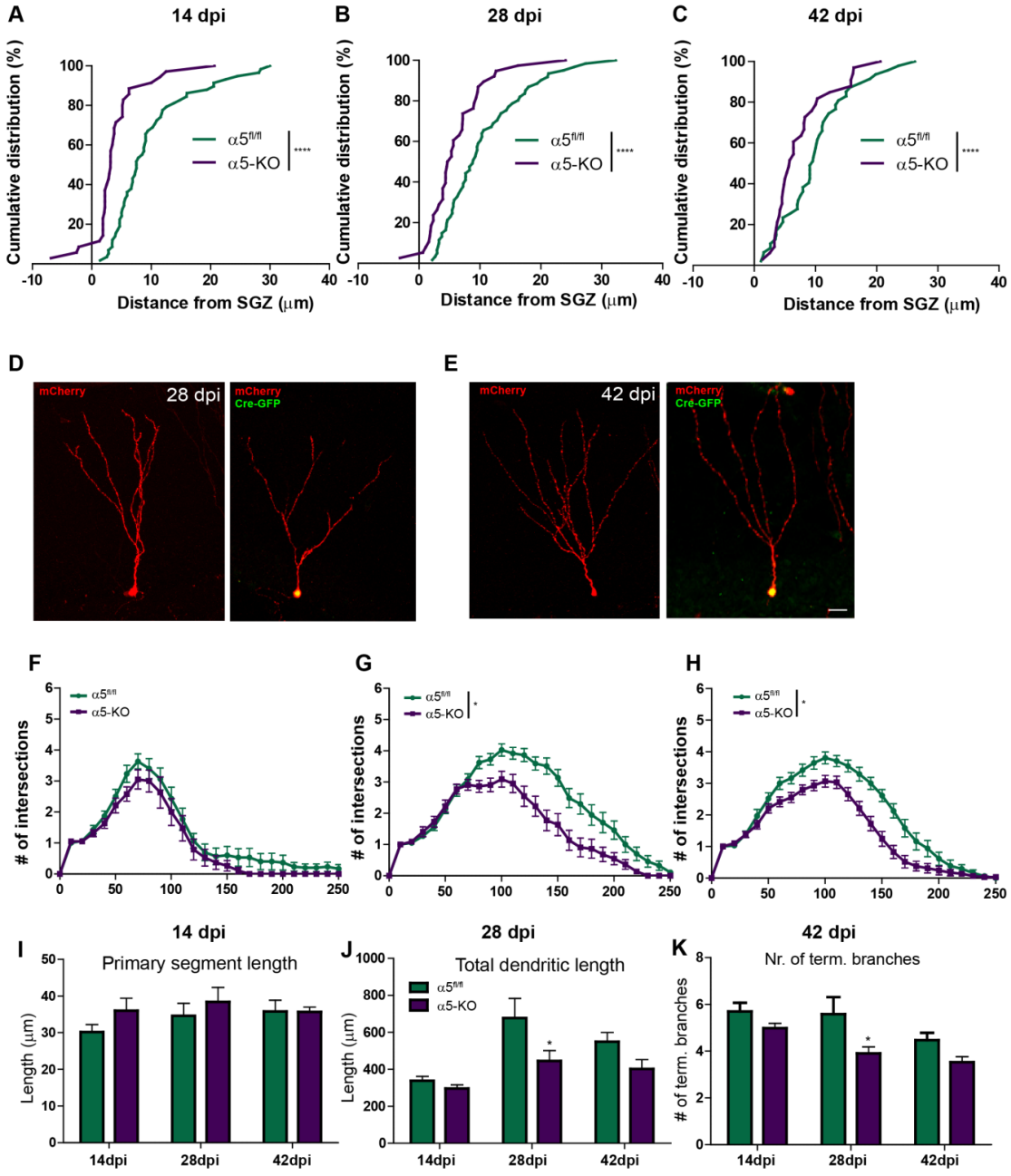

Figure 5 Dendritic development of newborn GCs in α5-floxed mice. ***A-C.*** Cumulative distribution analysis of the migration distance in the GCL of mRFP-positive ($\alpha5^{fl/fl}$) and mRFP/eGFP-positive (α5-KO) GCs at 14 (A), 28 (B) and 42 (C) dpi. At all three time-points a significantly reduced migrated distance was measured in double-positive GCs (Kolmogorov-Smirnov test: $P<0.0001$). ***D, E.*** Representative images showing dendritic arborization of $\alpha5^{fl/fl}$ and KO cells at 28 (D) and 42 (E) dpi. ***F-H.*** Quantification of dendritic arborization by Sholl analysis at 14 (F), 28 (G) and 42 (H) dpi. The number of intersections between eGFP-positive dendrite segments and virtual concentric lines centered on the cell body and spaced by 10 µm is depicted (mean SEM; *N*=3-6 mice per group). No difference in dendritic complexity was observed at 14 dpi. At 28 and 42 dpi, mRFP/eGFP-positive GCs revealed a significant reduction in dendritic complexity at the distal dendritic part (between 40 and 200 µm away from the soma). ***I-K.*** Analysis of dendritic morphology in $\alpha5^{fl/fl}$ and KO newborn GCs. Single-cell deletion of *Gabra5* resulted in a significant reduction in total dendritic length and number of terminal branches at 28 dpi (**P*<0.05; Bonferroni *post-hoc* tests). Scale bar: 20 µm.

Further, quantification of dendritic maturation by Sholl analysis (Fig. 5D,E) revealed no difference at 14 dpi between mutant and Cre-negative GCs, confirming our results above that α5-$GABA_A$Rs are not essential for initial dendritic growth (Fig. 5F). However, at 28 dpi, the dendritic arborization of mutant cells in the molecular layer was less complex compared to Cre-negative cells, as seen by comparing the area-under-the-curves (unpaired t-test, $t_{42} = 2.139$; $P = 0.0383$) (Fig. 5G). The difference remained evident at 42 dpi, in particular in the inner two-thirds of distal branches (on average, between 40 and 200 μm away from the soma) in mutant cells compared to Cre-negative cells (unpaired t-test, $t_{32} = 2.216$; $P = 0.0339$; Fig. 5H).

The remaining dendritic parameters measured (length of the primary segment, total length and number branches) exhibited similar differences as those seen between wild-type and α5-het mice. Thus, the length of the primary segment was not affected by the mutation (Fig. 5I), whereas statistical analysis of total dendritic length showed a significant effect of time ($F_{(2,12)} = 8.917$; $P = 0.0042$) and genotype ($F_{(1,12)} = 8.579$; $P = 0.0126$), but without an interaction. *Post-hoc* analysis revealed that mutant cells had a reduced total dendritic length at 28 dpi (Fig. 5J). Further, analysis of the number of terminal branches showed a significant effect of time ($F_{(2,12)} = 5.776$; $P = 0.0175$) and genotype ($F_{(1,12)} = 11.79$; $P = 0.0050$), but no interaction. Mutant cells had a reduced number of terminal branches at 28 dpi (Fig. 5K). The lower values obtained by quantifying mRFP-positive cells in C57Bl6/J mice compared to the eGFP-positive cells in wild-type and α5-mutant mice likely reflect the lower signal-to-noise ratio obtained with mRFP labeling; however, these should not affect the conclusion, since we compared mutant (Cre-positive) and Cre-negative cells in the same tissue sections.

Taken together, these data suggest that the effects seen in adult-born GCs of α5-het mice are due to cell-autonomous action of α5-$GABA_A$Rs controlling their migration and dendrite development. Intriguingly, the deficits were not more severe upon Cre-mediated inactivation of both *Gabra5* alleles, indicating that a partial deficit in α5-$GABA_A$Rs can cause a functional imbalance that is not worsened by the complete elimination of the receptor.

Discussion

To our knowledge, despite the intense scrutiny given to α5-$GABA_A$Rs for their role in regulating neuronal excitability and cognitive performances (see Introduction), their contribution to adult neurogenesis has not yet been investigated. The surprising lack of phenotype in global α5-KO mice might be explained best by the activation of compensatory mechanisms, notably an up-regulation of α1-, α3- and α4-$GABA_A$Rs. However, the results show that a partial reduction of α5-$GABA_A$Rs causes severe and enduring alterations of the migration and dendrite development of adult-born GCs. These effects are, for a large part, cell-autonomous, as shown by conditional deletion of *Gabra5* selectively in newborn GCs. Remarkably, despite the evidence that silencing α5-$GABA_A$Rs leads to hyper-excitability due to reduced tonic inhibition (Glykys and Mody, 2006, Bonin et al., 2007), we observed a deficit, rather than an excess, in dendrite growth and branching, raising the question of which effects α5-$GABA_A$Rs exert on developing cells under physiological conditions.

Effects of *Gabra5*-deletion in the dentate gyrus

In analogy with previous studies of targeted *Gabra1-Gabra4* gene deletions (see (Fritschy and Panzanelli, 2014) for review), and with previous reports on mice carrying a chromosomal deletion encompassing *Gabra5* (Fritschy et al., 1997, Fritschy et al., 1998), a key premise of the present work is that *Gabra5* targeting results in the complete loss of α5-$GABA_A$Rs. Given the low relative abundance of these receptors in the CNS, this effect would be difficult to demonstrate biochemically. Further, given the fact that the expression pattern of $GABA_A$R subunits by neural precursor cells is only poorly established, a major issue is whether inactivation of α5-$GABA_A$Rs affects newborn cells directly, or indirectly by changing the properties of neuronal networks in the DG (and its afferent/efferent connections). Finally, it is not known whether α5-$GABA_A$Rs possibly expressed in developing GCs are post- or extrasynaptic. In view of the convergent, but not identical, phenotype seen in α5-het mice and mutant $\alpha5^{fl/fl}$ GCs, we conclude that both direct and indirect effects contribute to the altered migration behavior and dendrite formation.

Accordingly, the lack of phenotype observed in α5-KO mice likely reflects compensatory changes affecting both the intrinsic properties of developing GCs and their environment.

Previous studies had already shown in the hippocampus that up-regulation of the δ subunit compensates, in part, for the loss of tonic inhibition mediated by α5-$GABA_AR$ (Glykys and Mody, 2006). However, this change was not sufficient to prevent network hyper-excitability in CA1. It is noteworthy that we found no increase in α2 subunit immunoreactivity in α5-KO mice. As α2-$GABA_ARs$ are the most abundant subtype in the hippocampal formation, this observation might explain the increased excitability of CA1 pyramidal cells and networks (Bonin et al., 2007).

It is not possible to determine whether the up-regulation of α1 and α4 subunit immunoreactivity detected in the DG also occurs in immature GCs. Furthermore, compensatory changes could affect many other molecules, notably voltage-gated ion channels (Brickley et al., 2001). However, since both α1 and α4 subunits contribute to extrasynaptic $GABA_ARs$ in the DG (Glykys et al., 2008, Herd et al., 2008), their global increase in the DG likely contributes to maintain tonic inhibition in α5-KO mice. Therefore, one might conclude from the lack of phenotype observed in the present study in adult-born GC migration and dendrite development, that adult neurogenesis is protected from major disturbances in α5-KO mice by activation of selective compensatory mechanisms. Given that the α5 subunit is strongly expressed during fetal development, the purpose of these compensatory mechanisms is likely to preserve proper CNS development.

The strong phenotype observed in α5-het mice, especially when compared to the full knockout, suggests that partial preservation of α5-$GABA_AR$ function is sufficient to prevent activation of compensatory mechanisms. This conclusion might explain why α5(H105R) mice, which only lack about 30% α5-$GABA_ARs$, exhibit multiple signs of hippocampus (and amygdala) dysfunction (see Introduction). One might speculate, therefore, that these knock-in mice also have a deficit in adult neurogenesis, which, in turn, could contribute to their altered, in part improved, cognitive abilities.

Effect of partial and single-cell deletion of α5-$GABA_ARs$

Disruption of tonic inhibition by the deletion of the α4-$GABA_ARs$ strongly affects neural precursor cell proliferation, as well as migration and dendritic development of newborn neurons (Duveau et al., 2011). In these cells, patch-clamp recordings performed at 14-21 dpi revealed a complete loss of tonic inhibition, indicating the prime role of α4-$GABA_ARs$ in developing GCs for mediating extrasynaptic effects of GABA.

Consequently, α5-$GABA_A$Rs might either be expressed later (which is unlikely), or contribute to slow or phasic inhibition in developing GCs. Ivy/neurogliaform cells are the main interneuron subtype that forms initial connections onto newborn GCs, and exert a form of slow transmission modulated by GABA transporters (Markwardt et al., 2011). While the $GABA_AR$ subtype mediated these effects has not been identified, α5-$GABA_A$Rs could be a likely candidate. The presence of α5-$GABA_AR$ in neural stem cells of the SGZ has been suggested in a study of conditional γ2-subunit gene deletion in mice, showing that a zolpidem-insensitive $GABA_AR$ subtype controls proliferation of quiescent radial glial-like NSCs and regulates differentiation of astrocytes (Song et al., 2012b). To fully understand the role of α5-$GABA_A$Rs in the SGZ, it will, therefore, be essential to determine their specific function and subcellular distribution.

The phenotype observed upon conditional *Gabra5* deletion either in α5-het mice and in mutant GCs of $\alpha5^{fl/fl}$ mice points to a cell-autonomous function of α5-$GABA_A$Rs, promoting neuronal development of newborn neurons, and, therefore, confirming their early expression. Failure to initiate proper migration, notably in the correct direction, underscores the importance of GABAergic transmission for positioning of adult-born neurons in the GCL. Previous evidence showed that this mechanism requires activation of Cdk5 (Jessberger et al., 2008a), making it plausible that signaling through α5-$GABA_A$Rs acts up-stream of Cdk5. This is further supported from evidence that Cdk5 activation is required for proper dendritic growth, via activation of focal adhesion kinase and phosphorylation of the semaphorin receptors neuropilin 1 and 2 (Ng et al., 2013). Inactivation of this pathway phenocopies the effects seen here in mutant GCs.

Nevertheless, considering that inactivation of *Gabra5* causes neuronal and network hyper-excitability, and presumably enhanced, rather than diminished Ca^{2+} signaling, it is puzzling that mutant cells lacking these receptors exhibit a strong and long-lasting impairment in dendritic development. This point is even further emphasized by the fact that the density of spines, representing sites of glutamatergic input, is not affected in mutant cells, suggesting that local depolarization from active spines might be buffered less effectively than in wild-type cells. This apparent paradox can be solved, hypothetically, by postulating that α5-$GABA_A$Rs, besides reducing intrinsic excitability by modulating membrane conductance, have a depolarizing action in adult-born GCs, allowing the opening of voltage-gated Ca^{2+} channels to activate intra-cellular signaling cascades. An obvious candidate would be Ca^{2+}-dependent activation of immediate early

genes (e.g., CREM, CREB, Arc, c-fos), which all contribute to maturation of newborn neurons (Berninger et al., 1995, Obrietan et al., 2002, Jagasia et al., 2009).

The less severe phenotype observed in mutant cells for $\alpha5^{fl/fl}$ mice compared to α5-het mice points to cell non-autonomous mechanisms regulating the maturation of adult-born neurons under the control of α5-$GABA_A$Rs. It is conceivable, of course, that enhanced network hyperexcitability might impair GABAergic control of neuronal maturation or disturb the balance between excitation and inhibition that is crucial for proper development and functional maturation of adult-born neurons (Dieni et al., 2012).

In conclusion, the present study demonstrates that a partial, but not a full, inactivation of α5-$GABA_A$Rs in mice causes enduring deficits in the maturation of adult-born GCs. When combined with the wealth of evidence that weakening α5-$GABA_A$R-mediated transmission improves cognitive performance, in particular in conditions of impaired excitatory/inhibitory balance, such as Down syndrome or Fragile X syndrome (reviewed in (Rudolph and Mohler, 2014)), these data shed a note of caution about possible negative consequences arising from chronic pharmacological treatment with negative allosteric modulator acting selectively on α5-$GABA_A$Rs.

Acknowledgements

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IV. GENERAL DISCUSSION

The fundamental role of GABAergic activity for proper development of adult-born neurons has been extensively studied in both neurogenic niches of the rodent brain. However, the underlying mechanisms activated by GABAergic transmission for regulating differentiation and synaptic integration of adult-born neurons are not yet fully understood. The aims of this thesis were to investigate whether gephyrin, acting as a signaling hub in the PSD of GABAergic synapses, rather than $GABA_AR$-mediated signals, regulates the differentiation and synaptic integration of adult-born OB neurons (Study I); and to establish how tonic GABAergic inhibition mediated by α5-$GABA_ARs$ influences the development of hippocampal newborn neurons (Study II). Together, these studies were expected to yield a better understanding of the specific roles of GABAergic signals and signaling cascades down-stream of the GABAergic PSD for the regulation of adult neurogenesis. In this chapter, I will first emphasize the main conclusions obtained in each study, and then discuss their implications in more detail.

The results of the first study revealed the crucial importance of gephyrin postsynaptic clustering for proper development of newborn neurons. In particular, we observed that disrupting gephyrin clustering at the GABAergic PSD by over-expressing the Geph(L2B) mutant construct strongly impairs dendritic growth and spine formation; conversely, constraining the phosphorylation status of gephyrin residue Ser270 resulted in preservation of plasticity mechanisms normally seen only in immature OB-GCs.

Strikingly, the deficit due to the over-expression of the Geph(L2B) mutant partially mirrored the phenotype seen after conditional deletion of the α2-$GABA_ARs$ in adult-born GCs (Pallotto et al., 2012), supporting the possibility that the effects of *Gabra2* silencing are due, in fact, to disruption of gephyrin clustering. Our ultrastructural investigation by electron microscopy revealed that over-expression of gephyrin (wild-type or mutant) has no detectable effect on the morphology of reciprocal synapses formed between GCs and M/T cells, despite the accumulation of eGFP-gephyrin in spines, presumably presynaptically. Altogether, our results strongly support the contention that the gephyrin scaffold serves as a hub for signaling cascades down-stream of GABAergic synapses involved in the regulation of neuronal maturation and spine formation.

In the second study, we investigated the functional role of α5-$GABA_AR$ in the regulation of different developmental steps of adult-born DG-GCs. Unexpectedly, a global deletion of the α5-$GABA_AR$ caused no obvious morphological alterations of adult-born GCs, whereas deletion of a single allele, presumably reducing α5-$GABA_ARs$ levels, had strong effects. However, we observed an up-regulation of α1-, α3- and α4-$GABA_ARs$ in distinct regions of the hippocampal formation of α5-KO mice, indicating the activation of potential compensatory mechanisms functionally replacing the missing α5-$GABA_ARs$. Importantly, a conditional deletion of *Gabra5* selectively in adult-born neurons only partially reproduced the effects seen in newborn neurons of α5-het mice, suggesting that the alterations in migration and dendrite formation are not only cell-autonomous.

Down-stream signaling of gephyrin scaffolding protein influencing maturation of adult-born GCs

Gephyrin is crucial for the clustering of $GABA_AR$ and GlyR at the synapse and facilitates their enrichment in the PSD (Fritschy et al., 2008). Thus, the absence of gephyrin results in the loss of $GABA_ARs$ at the synapse (Essrich et al., 1998, Kneussel et al., 1999). In turn, gephyrin depends on the presence of postsynaptic $GABA_ARs$ to form a cluster within the PSD of GABAergic synapses (Kralic et al., 2006, Patrizi et al., 2008, Panzanelli et al., 2011, Pallotto et al., 2012). This interdependence suggests that mechanisms influencing the postsynaptic clustering properties of gephyrin, in particular structural changes due to post-translational modifications, will have a strong impact on the efficacy of GABAergic transmission (reflecting the density of $GABA_ARs$ at the PSD) and might be key regulators of GABAergic synaptic plasticity. Our findings from Study I uncover an additional role of the postsynaptic gephyrin scaffold, by showing that it has the ability to modulate morphological development of neurons, most likely as a consequence of altered signaling down-stream of the synapse.

Previous work emphasized that ambient GABA acting through $GABA_ARs$ is central in the regulation of dendritic growth, by stabilization of lamelliopodia in newly generated interneurons of the OB (Gascon et al., 2006). Moreover, there is evidence that gephyrin scaffold properties play a role in the modulation of the cytoskeleton to regulate cell growth. In particular, blocking the activity of GSK3β and thereby abolishing gephyrin phosphorylation at Ser270, favors the formation of GABAergic synapses, as well as dendritic growth and branching of cultured neurons (Tyagarajan et al., 2011b, Rui et al., 2013, Tyagarajan et al., 2013) Therefore, the gephyrin scaffold might be the organizing structure of the PSD, which facilitates GABAergic

transmission and enables down-stream signaling acting on the cytoskeleton. This hypothesis is supported by the observation of Study I. Failure to form postsynaptic clusters, as seen in adult-born OB-GCs over-expressing eGFP-Geph(L2B), induced a structural deficit, whereas over-expression of eGFP-Geph(S270A) favored structural plasticity of adult-born GCs, leading to enhanced dendritic growth and spine formation.

Gephyrin interacts with various signaling molecules, like CB or Cdc42, which have the ability to modulate GABAergic transmission, synapse stability, and cytoskeleton dynamics (Xiang et al., 2006, Tyagarajan et al., 2011b, Mayer et al., 2013). Beside the important role of small GTPases, like Cdc42, RhoA and Rac1 in various cellular functions such as proliferation, cell migration, growth and survival, several studies indicated their relevance in the context of adult neurogenesis (Jaffe and Hall, 2005, Vadodaria et al., 2013). Knockdown of Cdc42 and Rac1 activity in adult-born DG neurons results in impaired dendritic development and spine morphogenesis (Vadodaria and Jessberger, 2013). These results suggest that Cdc42 and Rac1 are signaling proteins acting down-stream of gephyrin, possibly being activated by a GEF bound the gephyrin scaffold. PAK proteins, known to act as key regulators for cytoskeleton dynamics, are down-stream targets of Rac1 and Cdc42 (Kreis and Barnier, 2009). In addition, Rac1 is a down-stream effector of the GIT1/βPIX complex which plays a central role in the formation of dendritic spines at excitatory synapses and was previously shown to be essential for the maintenance of $GABA_A$Rs and gephyrin at the synapse by stabilizing F-actin filaments through PAK (Zhang et al., 2005, Smith et al., 2014). Therefore, the impaired dendritic growth upon the over-expression of eGFP-Geph(L2B) in adult-born GCs might be due to the disruption of the signaling cascade through CB, Cdc42, and PAK. In contrast, the increased dendritic branching and induction of transient spines upon eGFP-Geph(S270A) over-expression might be due to a long-term activation of the GIT1/βPIX/Rac1/PAK complex, contributing to increased cytoskeleton plasticity (Smith et al., 2014).

Moreover, Cdc42 forms a complex together with IQGAP1 protein, known to be involved in the regulation of the actin cytoskeleton (Briggs and Sacks, 2003). In the absence of Ca^{2+}, this complex binds and stabilizes F-actin, whereas increased Ca^{2+} concentration leads to the dissociation of the complex, enhancing cytoskeleton dynamics. In summary, the ability of gephyrin to interact with numerous signaling molecules at the GABAergic PSD supports the assumption that the gephyrin scaffold activates various down-stream pathways that regulate cytoskeleton dynamics.

An additional, or alternative, mechanism to explain our results involves dysregulation of Ca^{2+}-signaling. We have shown that over-expression of Geph(L2B) mutant precludes the formation of GABAergic synapses and spines. Since spines are eventually the recipient of glutamatergic synapses, one might expect a deficit in spine formation to cause reduced excitatory transmission, which might consequently affect Ca^{2+} influx and signaling. It is well known that intracellular Ca^{2+} is a second messenger activating various protein kinases, phosphatases, and proteases, including calpain-1, calmodulin, calcineurin and calmodulin-dependent protein kinase II (CaMKII), which are key effectors of Ca^{2+} -dependent signaling pathways. Moreover, LTP and long-term depression (LTD) modify synaptic plasticity and consequently the dynamics of the cytoskeleton, as well (Honkura et al., 2008, Gordon-Weeks and Fournier, 2014).Therefore, over-expression of Geph(L2B) mutant might reduce excitation by influencing glutamatergic synapses and their associated Ca^{2+} signaling. In other words, gephyrin may have the ability to modulate either structural- or synaptic plasticity by affecting Ca^{2+} signaling.
Activation of Ca^{2+} signaling cascades might be an alternative explanation for the formation of transient spines due to the over-expression of Geph(S270A) mutant. It is likely that an enhanced intracellular Ca^{2+} level induced by glutamatergic transmission either from spines in the GCL or from reciprocal synapses formed between GCs and M/T cells in the EPL induces the formation of new spines, accompanied by densely packed gephyrin clusters forming a GABAergic terminal.

Moreover, development of adult-born GCs is highly dependent on various transcription factors which are mostly activated via Ca^{2+} (reviewed in (Hodge and Hevner, 2011)). It is likely that transient Ca^{2+} influx induced either by GABAergic or glutamatergic activity in adult-born OB-GCs leads to the activation of cAMP response element-binding (CREB) protein, which is important for neuronal maturation, morphological differentiation and survival (Obrietan et al., 2002, Jagasia et al., 2009, Giachino et al., 2005, Herold et al., 2011). The curtailed survival rate of adult-born GCs expressing Geph(L2B) mutant, suggests that in those cells Ca^{2+} influx is highly diminished, resulting in a sparse activation of CREB.

Altogether, the results of Study I provide new insight in the role of gephyrin as a signaling molecule in the GABAergic PSD modulating structural plasticity and long-term survival in developing neurons. Especially, post-translational modification of gephyrin at residue Ser270 represents a mechanism which facilitates dendritic growth and maintains adult-born neurons in a

state of immature plasticity, allowing the formation of transient GABAergic synapses on spines. In contrast, a dominant-negative gephyrin isoform causes severe impairments in signaling cascades precluding the development of adult-born GCs.

Targeted *Gabra5*-deletion affects signaling pathways regulating morphological development

The α5-$GABA_A$Rs are anchored by radixin in the plasma membrane and are to a large extend extra-synaptically located, mediating tonic inhibition (Picard-Riera et al., 2002, Caraiscos et al., 2004, Loebrich et al., 2006, Glykys et al., 2008, Belelli et al., 2009). Radixin, an actin-binding protein with an F-actin binding motif in its C-terminal, is essential for the localization of α5-$GABA_A$Rs in the plasma membrane and connects the receptor with the cytoskeleton (Loebrich et al., 2006). Nevertheless, a small fraction of α5-$GABA_A$Rs are synaptically located and mediate slow phasic inhibition (Glykys and Mody, 2006, Zarnowska et al., 2009, Vargas-Caballero et al., 2010). The function of α5-$GABA_A$Rs has been extensively studied in the context of cognitive processes (Hauser et al., 2005, Prut et al., 2010) following the demonstration that reducing their function either genetically or pharmacologically with selective negative modulators improves learning and memory performance in selected hippocampus-dependent behavioral tests (Crestani et al., 2002, Yee et al., 2004, Dawson et al., 2006). Nowadays, the α5-$GABA_A$R is a key target for improving cognition in schizophrenia and cognitive disability, including Fragile X syndrome and Down syndrome (Rudolph and Mohler, 2014).

The main outcome of study II is that a partial, but not a global, deletion of the *Gabra5* gene (α5-het mice) or a conditional *Gabra5* deletion in adult-born DG-GCs of $\alpha5^{fl/fl}$ mice, results in severe alterations of migration and dendritic growth. In analogy with the conclusions of Study I, it is possible that the reduction of α5-$GABA_A$Rs and perhaps also radixin, contributes to a disruption of the actin cytoskeleton dynamics, leading to the observed phenotypes. Alternatively, or in addition, it is also conceivable that the α5-$GABA_A$Rs activate down-stream signaling cascades contributing to morphological development of newborn neurons. A possible such candidate, in line with the phenotype seen in α5-het and $\alpha5^{fl/fl}$ mice, is cyclin-dependent kinase 5 (Cdk5), known to be critically involved in the migration, neuronal maturation, integration, and survival of adult-born neurons (reviewed in (Jessberger et al., 2009)). Reduced Cdk5 activity in adult-born DG-GCs causes aberrant neural migration and reduction in dendritic growth (Jessberger et al., 2008a). Cdk5 interacts with different proteins of the small RhoGTPases family, like Cdc42

and Rac1, which in turn interact with PAK (Nikolic et al., 1998). This signaling cascade is likely to have an impact on the reorganization of the actin cytoskeleton, and thereby on the neural migration and morphological development.

Recently, it has been demonstrated that Cdk5 activity is necessary for correct migration and dendritic maturation, via activation of focal adhesion kinase (FAK) and phosphorylation of the semaphorin receptors neuropilin 1 and 2. Previous work has demonstrated the critical role of FAK for axon collapse and dendritic growth (Schlomann et al., 2009). Single cell ablation of neuropilin 1 or 2 in adult-born GCs causes a reduction in cell migration, dendritic length and branching (Ng et al., 2013). Because this phenotype is reminiscent of the effects induced by targeted deletion of the α5-GABA$_A$R, it is possible that α5-GABA$_A$R-mediated transmission activates down-stream signaling cascades via Cdk5 and the FAK/neuropilin 1/2 signaling pathway.

In addition, Cdk5 has the ability to regulate ERK1/2 and Akt activity, promoting neuronal survival (Li et al., 2003, Kesavapany et al., 2004). Although α5-het mice have only a reduced level of α5-GABA$_A$Rs, these GCs exhibited stronger impairments in maturation and dendrite formation between 28 and 42 dpi compared to mutant $\alpha5^{fl/fl}$ GCs. This observation suggests that a fraction of mutant $\alpha5^{fl/fl}$ GCs affected by severe migration and growth deficits died between 28 and 42 dpi. Therefore, the difference in dendritic complexity was reduced compared to α5-het mice due to selective survival of less affected GCs. This hypothesis is based upon the possibility of an imbalance in the ERK1/2, Akt pathways mediated via Cdk5 signaling.

In immature adult-born DG-GCs, GABAergic activity causes a depolarizing action, leading to an increase in intracellular Ca^{2+} concentration. In turn, Ca^{2+} signaling might regulate migration and maturation, most probably through the activation of immediate early genes (e.g. CREB, CREM, Arc, c-fos). In particular, Jagasia et al. have shown that phosphorylation of CREB, mediated through the depolarizing action of GABA, promotes development and survival of adult-born GCs (Jagasia et al., 2009, Magill et al., 2010). Considering that inactivation of the *Gabra5* gene causes an increase in neuronal excitability and network activity in CA1 pyramidal cells, presumably accompanied by elevated intracellular Ca^{2+} concentration, the phenotype of adult-born GCs in α5-het and mutant $\alpha5^{fl/fl}$ GCs at 28, respectively, 42 dpi was unexpected. Furthermore, it is known that GCs undergo a critical period with enhanced LTP between 28 and 42 dpi (Ge et al., 2007). In this time-window, the transition of GABA from excitatory to

inhibitory occurs (Ge et al., 2006), which leads to an increased synaptic plasticity in adult-born GCs due to lower threshold activation upon changes in network activity (Marin-Burgin et al., 2012). It is conceivable that abolishing *Gabra5* disturbed the balance between E/I, leading to a strong elevation of intracellular Ca^{2+} concentration, which might cause neurotoxicity or, as mentioned above, instability of the survival pathway via ERK1/2 or Akt.

Tonic inhibition of mature hippocampal GCs is prevalently mediated by δ-$GABA_A$Rs, likely associated with the α4 subunit (Glykys et al., 2008). Adult-born GCs in α4-KO mice have a complete loss of tonic inhibition at 15-21 dpi, accompanied with alterations in migration and dendrite growth. Therefore, the phenotype observed upon partial inactivation of the α5-$GABA_A$R was unexpectedly strong, raising the possibility that α5-$GABA_A$Rs in adult-born GCs might contribute to phasic transmission. This possibility would be supported by the properties of the first GABAergic synapses formed onto newborn GCs, which have the characteristics of slow inhibitory transmission (Glykys and Mody, 2006, Zarnowska et al., 2009, Vargas-Caballero et al., 2010). Electron microscopy experiments have suggested the presence of α5-$GABA_A$Rs in some GABAergic synapses, colocalized with gephyrin (Serwanski et al., 2006). One might speculate, therefore, that synaptic α5-$GABA_A$Rs interacting with gephyrin may contribute to cytoskeleton dynamics. Electrophysiological recordings from adult-born GCs in α5-het mice will be required to verify this possibility.

Beyond adult neurogenesis

During mammalian CNS development and in the adult brain, as well, GABAergic signaling has an essential role in the regulation of neural proliferation, migration, differentiation, and neural network wiring, while keeping the balance between excitation and inhibition. The effects of GABAergic transmission are heterogeneous, as determined by the functional properties of the main $GABA_A$R subtypes, differing in subunit composition, subcellular localization, and spatio-temporal expression. Further, they are determined by the Cl^- gradients established by KCC2 and NKCC1.

In both studies, we examined the functional role of GABAergic synapse plasticity in the context of adult neurogenesis. However, as Study I and Study II were performed in two different neurogenic regions, the question remains, whether the two systems share common mechanisms in mediating the development of adult-born GCs. Although GABAergic OB-GC and glutamatergic DG-GCs have very different properties and synaptic connections, we show here

that early alterations in GABAergic transmission or synapse structure cause aberrant development, including deficits in migration, dendritic growth and arborization, and spine density. Therefore, the results of both studies indicate that GABAergic transmission is linked to the activity of signaling molecules which act on structural plasticity. The activation chain might be either direct, mediated by specific receptors, and/or indirectly through the gephyrin scaffold which possibly anchors effector molecules (see above and Introduction). It is conceivable that Ca^{2+}-dependent mechanisms play a role, either induced via the formation of excitatory synapses, or through the control of GABAergic or glutamatergic activity. It is known that OB-GCs express to a large extent the α5-$GABA_AR$, and gephyrin is also post-synaptically located at GABAergic synapses in DG-GCs. It is, therefore, likely that the mechanisms described here can be applied to both systems, despite the two different types of neurons.

Beyond this, the question arises what our findings mean in the context of brain diseases. Alterations of GABAergic signaling have been reported in a range of neurodevelopmental diseases, such as autism, Down syndrome and schizophrenia, as well as mood disorders and epilepsy (Deidda et al., 2014). However, it is still unclear how dysregulation of GABAergic transmission and plasticity contributes to distinct brain pathologies. Nevertheless, there are studies showing that changes in inhibitory circuits are linked to specific defects in the development and function of GABAergic interneurons. GABAergic interneurons primarily regulate and synchronize the activity of principal cells. It has been reported that deficits in cortical inhibition due to abnormal interneuron function in the prefrontal cortex from patients with schizophrenia and depression might underlie impaired cognitive functions (Lewis et al., 2005, Luscher et al., 2011b, Marin-Burgin et al., 2012). In particular, in mouse models and patients with schizophrenia, the function and connectivity of PV-expressing interneurons are altered, in part secondarily to alterations in gene expression that control the development of PV^+ interneurons, such as DISC1 or ERBB4 (Lewis et al., 2005, Marin-Burgin et al., 2012). In addition, these cells control the occurrence and timing of critical periods of brain development and are, therefore, critically involved in the emergence of proper sensory and executive functions of the neocortex (Hensch, 2005, Timofeeva and Levin, 2011).
Moreover, studies from animal models as well as from patients reported changes occurring at the level of $GABA_AR$ subtype expression, where disinhibition causes pathological hyper-excitability (reviewed in (Fritschy and Brunig, 2003, Luscher et al., 2011b, Deidda et al., 2014)). In particular, partial deletion of the γ2 subunit gene between the second and fifth postnatal week

induced anxiety-depression-like phenotype (Shen et al., 2012). Furthermore, these mice exhibited curtailed survival and deficits in dendrite and spine maturation of adult-born hippocampal neurons, whereas the proliferation rate was unaffected (Ren et al., 2014). Despite these studies, the causality is still largely unknown.

BZs have been commonly used to treat disorders resulting from network hyper-excitability. In mouse models of Down syndrome, which show impaired spatial and recognition memory and enhanced LTD, administration of non-competitive $GABA_AR$ antagonists has been used to treat increased GABAergic transmission and cognitive impairments (Fernandez et al., 2007). But due to the high risk of seizure induction, medication with $GABA_AR$ antagonist in patients with Down syndrome is unsuitable. Rather, weakening of α5-$GABA_AR$-mediated transmission with a selective allosteric modulator rescues cognitive performance (reviewed in (Rudolph and Mohler, 2014)). In connection with this pharmacological approach, our findings from study II raise a note of caution.

The impairments in the development of adult-born DG-GC in α5-het mice suggest that chronic use of negative allosteric modulators acting on α5-$GABA_ARs$ might have deleterious consequences in human.

As mentioned above, impaired function of interneurons or altered expression profiles of $GABA_ARs$ have been implicated in various neurological diseases, while the role of the interplay between different members of the PSD in GABAergic synapses remains largely unknown. It has been shown that a mutation in the CB gene (ARHGEF9) leading to a loss-of-function, is responsible for X-linked mental retardation associated with epilepsy due to impaired $GABA_AR$ clustering (Shimojima et al., 2011). Furthermore, in human brain from mesial temporal epilepsy patient, pathological gephyrin expression was identified. These patients exhibited curtailed postsynaptic gephyrin and α2-$GABA_AR$ clustering, due to abnormally spliced gephyrin variants in the G domain known to be important for its self oligomerization (Forstera et al., 2010). Taken together with our present results, these observations underscore the relevance of the GABAergic PSD for normal brain development and function.

Although pharmacological treatments targeting $GABA_ARs$ provide symptomatic relief of anxiety, sleep disorders and epileptic seizures, the consequences on down-stream signaling cascades are largely elusive. With this work, we demonstrate that GABAergic transmission is able to regulate cell growth and structural plasticity by modulating the cytoskeleton. Our first

study revealed that regulation of the gephyrin scaffold by intracellular signaling cascades coordinates neuronal development and synaptic plasticity, suggesting that a wide diversity of signals can impinge on GABAergic function. The second study demonstrates the importance of extra-synaptic receptors, like α5-$GABA_A$Rs, during neuronal maturation, and thereby underscores the importance of global, tonic regulation of neuronal excitability for ensuring proper formation of neuronal circuits.

REFERENCES

Abrous DN, Koehl M, Le Moal M (2005) Adult neurogenesis: from precursors to network and physiology. Physiological reviews 85:523-569.

Alonso M, Lepousez G, Sebastien W, Bardy C, Gabellec MM, Torquet N, Lledo PM (2012) Activation of adult-born neurons facilitates learning and memory. Nature neuroscience 15:897-904.

Alonso M, Viollet C, Gabellec MM, Meas-Yedid V, Olivo-Marin JC, Lledo PM (2006) Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:10508-10513.

Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD (2001) A unified hypothesis on the lineage of neural stem cells. Nature reviews Neuroscience 2:287-293.

Alvarez-Buylla A, Lim DA (2004) For the long run: maintaining germinal niches in the adult brain. Neuron 41:683-686.

Bang ML, Owczarek S (2013) A matter of balance: role of neurexin and neuroligin at the synapse. Neurochemical research 38:1174-1189.

Baudouin S, Scheiffele P (2010) SnapShot: Neuroligin-neurexin complexes. Cell 141:908, 908 e901.

Belaidi AA, Schwarz G (2013) Molybdenum cofactor deficiency: metabolic link between taurine and S-sulfocysteine. Advances in experimental medicine and biology 776:13-19.

Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (2009) Extrasynaptic GABAA receptors: form, pharmacology, and function. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:12757-12763.

Benarroch EE (2013) Adult neurogenesis in the dentate gyrus: general concepts and potential implications. Neurology 81:1443-1452.

Bergmann O, Liebl J, Bernard S, Alkass K, Yeung MS, Steier P, Kutschera W, Johnson L, Landen M, Druid H, Spalding KL, Frisen J (2012) The age of olfactory bulb neurons in humans. Neuron 74:634-639.

Berninger B, Marty S, Zafra F, da Penha Berzaghi M, Thoenen H, Lindholm D (1995) GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro. Development 121:2327-2335.

Bolteus AJ, Bordey A (2004) GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:7623-7631.

Bonaguidi MA, Wheeler MA, Shapiro JS, Stadel RP, Sun GJ, Ming GL, Song H (2011) In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell 145:1142-1155.

Bonin RP, Martin LJ, MacDonald JF, Orser BA (2007) Alpha5GABAA receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. Journal of neurophysiology 98:2244-2254.

Bourgeron T (2009) A synaptic trek to autism. Current opinion in neurobiology 19:231-234.

Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. Nature 409:88-92.

Briggs MW, Sacks DB (2003) IQGAP1 as signal integrator: Ca2+, calmodulin, Cdc42 and the cytoskeleton. FEBS letters 542:7-11.

Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175-187.

Budreck EC, Scheiffele P (2007) Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. The European journal of neuroscience 26:1738-1748.

Cameron HA, McKay RD (2001) Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. The Journal of comparative neurology 435:406-417.

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proceedings of the National Academy of Sciences of the United States of America 101:3662-3667.

Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM (2003) Becoming a new neuron in the adult olfactory bulb. Nature neuroscience 6:507-518.

Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) GABAA receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proceedings of the National Academy of Sciences of the United States of America 103:15230-15235.

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:5572-5580.

Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Bluthmann H, Mohler H, Rudolph U (2002) Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proceedings of the National Academy of Sciences of the United States of America 99:8980-8985.

Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR (2006) An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. The Journal of pharmacology and experimental therapeutics 316:1335-1345.

Deidda G, Bozarth IF, Cancedda L (2014) Modulation of GABAergic transmission in development and neurodevelopmental disorders: investigating physiology and pathology to gain therapeutic perspectives. Frontiers in cellular neuroscience 8:119.

Dejanovic B, Semtner M, Ebert S, Lamkemeyer T, Neuser F, Luscher B, Meier JC, Schwarz G (2014) Palmitoylation of gephyrin controls receptor clustering and plasticity of GABAergic synapses. PLoS biology 12:e1001908.

Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nature reviews Neuroscience 11:339-350.

Dieni CV, Chancey JH, Overstreet-Wadiche LS (2012) Dynamic functions of GABA signaling during granule cell maturation. Frontiers in neural circuits 6:113.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703-716.

Dong N, Qi J, Chen G (2007) Molecular reconstitution of functional GABAergic synapses with expression of neuroligin-2 and GABAA receptors. Molecular and cellular neurosciences 35:14-23.

Duveau V, Laustela S, Barth L, Gianolini F, Vogt KE, Keist R, Chandra D, Homanics GE, Rudolph U, Fritschy JM (2011) Spatiotemporal specificity of GABAA receptor-mediated regulation of adult hippocampal neurogenesis. The European journal of neuroscience 34:362-373.

Eichenbaum H, Mathews P, Cohen NJ (1989) Further studies of hippocampal representation during odor discrimination learning. Behavioral neuroscience 103:1207-1216.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nature medicine 4:1313-1317.

Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B (1998) Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. Nature neuroscience 1:563-571.

Feierstein CE (2012) Linking adult olfactory neurogenesis to social behavior. Frontiers in neuroscience 6:173.

Feng G, Tintrup H, Kirsch J, Nichol MC, Kuhse J, Betz H, Sanes JR (1998) Dual requirement for gephyrin in glycine receptor clustering and molybdoenzyme activity. Science 282:1321-1324.

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC (2007) Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nature neuroscience 10:411-413.

Forstera B, Belaidi AA, Juttner R, Bernert C, Tsokos M, Lehmann TN, Horn P, Dehnicke C, Schwarz G, Meier JC (2010) Irregular RNA splicing curtails postsynaptic gephyrin in the cornu ammonis of patients with epilepsy. Brain : a journal of neurology 133:3778-3794.

Fritschy JM, Benke D, Johnson DK, Mohler H, Rudolph U (1997) GABAA-receptor alpha-subunit is an essential prerequisite for receptor formation in vivo. Neuroscience 81:1043-1053.

Fritschy JM, Brunig I (2003) Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacology & therapeutics 98:299-323.

Fritschy JM, Harvey RJ, Schwarz G (2008) Gephyrin: where do we stand, where do we go? Trends in neurosciences 31:257-264.

Fritschy JM, Johnson DK, Mohler H, Rudolph U (1998) Independent assembly and subcellular targeting of GABA(A)-receptor subtypes demonstrated in mouse hippocampal and olfactory neurons in vivo. Neuroscience letters 249:99-102.

Fritschy JM, Mohler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. The Journal of comparative neurology 359:154-194.

Fritschy JM, Panzanelli P (2014) GABAA receptors and plasticity of inhibitory neurotransmission in the central nervous system. The European journal of neuroscience 39:1845-1865.

Fritschy JM, Paysan J, Enna A, Mohler H (1994) Switch in the expression of rat GABAA-receptor subtypes during postnatal development: an immunohistochemical study. The Journal of neuroscience : the official journal of the Society for Neuroscience 14:5302-5324.

Frotscher M, Drakew A, Heimrich B (2000) Role of afferent innervation and neuronal activity in dendritic development and spine maturation of fascia dentata granule cells. Cereb Cortex 10:946-951.

Gage FH (2000) Mammalian neural stem cells. Science 287:1433-1438.

Gallarda BW, Lledo PM (2012) Adult neurogenesis in the olfactory system and neurodegenerative disease. Current molecular medicine 12:1253-1260.

Gascon E, Dayer AG, Sauvain MO, Potter G, Jenny B, De Roo M, Zgraggen E, Demaurex N, Muller D, Kiss JZ (2006) GABA regulates dendritic growth by stabilizing lamellipodia in newly generated interneurons of the olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:12956-12966.

Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439:589-593.

Ge S, Yang CH, Hsu KS, Ming GL, Song H (2007) A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 54:559-566.

Ghosh S, Larson SD, Hefzi H, Marnoy Z, Cutforth T, Dokka K, Baldwin KK (2011) Sensory maps in the olfactory cortex defined by long-range viral tracing of single neurons. Nature 472:217-220.

Giachino C, De Marchis S, Giampietro C, Parlato R, Perroteau I, Schutz G, Fasolo A, Peretto P (2005) cAMP response element-binding protein regulates differentiation and survival of newborn neurons in the olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:10105-10118.

Gibson JR, Huber KM, Sudhof TC (2009) Neuroligin-2 deletion selectively decreases inhibitory synaptic transmission originating from fast-spiking but not from somatostatin-positive interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:13883-13897.

Gill KM, Grace AA (2014) The role of alpha5 GABAA receptor agonists in the treatment of cognitive deficits in schizophrenia. Current pharmaceutical design 20:5069-5076.

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? The Journal of neuroscience : the official journal of the Society for Neuroscience 28:1421-1426.

Glykys J, Mody I (2006) Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. Journal of neurophysiology 95:2796-2807.

Gordon-Weeks PR, Fournier AE (2014) Neuronal cytoskeleton in synaptic plasticity and regeneration. Journal of neurochemistry 129:206-212.

Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 119:1013-1026.

Gross CG (2000) Neurogenesis in the adult brain: death of a dogma. Nature reviews Neuroscience 1:67-73.

Guillery RW (2000) Early electron microscopic observations of synaptic structures in the cerebral cortex: a view of the contributions made by George Gray (1924-1999). Trends in neurosciences 23:594-598.

Harvey K, Duguid IC, Alldred MJ, Beatty SE, Ward H, Keep NH, Lingenfelter SE, Pearce BR, Lundgren J, Owen MJ, Smart TG, Luscher B, Rees MI, Harvey RJ (2004) The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:5816-5826.

Hauser J, Rudolph U, Keist R, Mohler H, Feldon J, Yee BK (2005) Hippocampal alpha5 subunit-containing GABAA receptors modulate the expression of prepulse inhibition. Molecular psychiatry 10:201-207.

Hensch TK (2005) Critical period plasticity in local cortical circuits. Nature reviews Neuroscience 6:877-888.

Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D (2008) The expression of GABAA beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. The Journal of physiology 586:989-1004.

Herold S, Jagasia R, Merz K, Wassmer K, Lie DC (2011) CREB signalling regulates early survival, neuronal gene expression and morphological development in adult subventricular zone neurogenesis. Molecular and cellular neurosciences 46:79-88.

Herweg J, Schwarz G (2012) Splice-specific glycine receptor binding, folding, and phosphorylation of the scaffolding protein gephyrin. The Journal of biological chemistry 287:12645-12656.

Hodge RD, Hevner RF (2011) Expression and actions of transcription factors in adult hippocampal neurogenesis. Developmental neurobiology 71:680-689.

Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GC, Kasai H (2008) The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 57:719-729.

Hoon M, Bauer G, Fritschy JM, Moser T, Falkenburger BH, Varoqueaux F (2009) Neuroligin 2 controls the maturation of GABAergic synapses and information processing in the retina. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:8039-8050.

Hoon M, Soykan T, Falkenburger B, Hammer M, Patrizi A, Schmidt KF, Sassoe-Pognetto M, Lowel S, Moser T, Taschenberger H, Brose N, Varoqueaux F (2011) Neuroligin-4 is localized to glycinergic postsynapses and regulates inhibition in the retina. Proceedings of the National Academy of Sciences of the United States of America 108:3053-3058.

Jaffe AB, Hall A (2005) Rho GTPases: biochemistry and biology. Annual review of cell and developmental biology 21:247-269.

Jagasia R, Steib K, Englberger E, Herold S, Faus-Kessler T, Saxe M, Gage FH, Song H, Lie DC (2009) GABA-cAMP response element-binding protein signaling regulates maturation and survival of newly generated neurons in the adult hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:7966-7977.

Jessberger S, Aigner S, Clemenson GD, Jr., Toni N, Lie DC, Karalay O, Overall R, Kempermann G, Gage FH (2008a) Cdk5 regulates accurate maturation of newborn granule cells in the adult hippocampus. PLoS biology 6:e272.

Jessberger S, Gage FH, Eisch AJ, Lagace DC (2009) Making a neuron: Cdk5 in embryonic and adult neurogenesis. Trends in neurosciences 32:575-582.

Jessberger S, Toni N, Clemenson GD, Jr., Ray J, Gage FH (2008b) Directed differentiation of hippocampal stem/progenitor cells in the adult brain. Nature neuroscience 11:888-893.

Kalscheuer VM, Musante L, Fang C, Hoffmann K, Fuchs C, Carta E, Deas E, Venkateswarlu K, Menzel C, Ullmann R, Tommerup N, Dalpra L, Tzschach A, Selicorni A, Luscher B, Ropers HH, Harvey K, Harvey RJ (2009) A balanced chromosomal translocation disrupting ARHGEF9 is associated with epilepsy, anxiety, aggression, and mental retardation. Human mutation 30:61-68.

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic GABAA receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. The European journal of neuroscience 32:1868-1888.

Kelsch W, Li Z, Eliava M, Goengrich C, Monyer H (2012) GluN2B-containing NMDA receptors promote wiring of adult-born neurons into olfactory bulb circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:12603-12611.

Kelsch W, Lin CW, Mosley CP, Lois C (2009) A critical period for activity-dependent synaptic development during olfactory bulb adult neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:11852-11858.

Kelsom C, Lu W (2013) Development and specification of GABAergic cortical interneurons. Cell & bioscience 3:19.

Kempermann G, Gage FH (1999) New nerve cells for the adult brain. Scientific American 280:48-53.

Kempermann G, Jessberger S, Steiner B, Kronenberg G (2004) Milestones of neuronal development in the adult hippocampus. Trends in neurosciences 27:447-452.

Kesavapany S, Amin N, Zheng YL, Nijhara R, Jaffe H, Sihag R, Gutkind JS, Takahashi S, Kulkarni A, Grant P, Pant HC (2004) p35/cyclin-dependent kinase 5 phosphorylation of ras guanine nucleotide releasing factor 2 (RasGRF2) mediates Rac-dependent Extracellular Signal-regulated kinase 1/2 activity, altering RasGRF2 and microtubule-associated protein 1b distribution in neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:4421-4431.

Kesner RP (2007) Behavioral functions of the CA3 subregion of the hippocampus. Learn Mem 14:771-781.

Kins S, Betz H, Kirsch J (2000) Collybistin, a newly identified brain-specific GEF, induces submembrane clustering of gephyrin. Nature neuroscience 3:22-29.

Kirsch J, Langosch D, Prior P, Littauer UZ, Schmitt B, Betz H (1991) The 93-kDa glycine receptor-associated protein binds to tubulin. The Journal of biological chemistry 266:22242-22245.

Klausberger T (2009) GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. The European journal of neuroscience 30:947-957.

Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science 321:53-57.

Kneussel M, Brandstatter JH, Gasnier B, Feng G, Sanes JR, Betz H (2001) Gephyrin-independent clustering of postsynaptic GABA(A) receptor subtypes. Molecular and cellular neurosciences 17:973-982.

Kneussel M, Brandstatter JH, Laube B, Stahl S, Muller U, Betz H (1999) Loss of postsynaptic GABA(A) receptor clustering in gephyrin-deficient mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:9289-9297.

Kralic JE, Ledergerber DA, Fritschy JM (2005) Disruption of the neurogenic potential of the dentate gyrus in a mouse model of temporal lobe epilepsy with focal seizures. The European journal of neuroscience 22:1916-1927.

Kralic JE, Sidler C, Parpan F, Homanics GE, Morrow AL, Fritschy JM (2006) Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice. The Journal of comparative neurology 495:408-421.

Kreis P, Barnier JV (2009) PAK signalling in neuronal physiology. Cellular signalling 21:384-393.

Kriegstein AR, Noctor SC (2004) Patterns of neuronal migration in the embryonic cortex. Trends in neurosciences 27:392-399.

Kuhse J, Kalbouneh H, Schlicksupp A, Mukusch S, Nawrotzki R, Kirsch J (2012) Phosphorylation of gephyrin in hippocampal neurons by cyclin-dependent kinase CDK5 at Ser-270 is dependent on collybistin. The Journal of biological chemistry 287:30952-30966.

Lagier S, Carleton A, Lledo PM (2004) Interplay between local GABAergic interneurons and relay neurons generates gamma oscillations in the rat olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:4382-4392.

Lagier S, Panzanelli P, Russo RE, Nissant A, Bathellier B, Sassoe-Pognetto M, Fritschy JM, Lledo PM (2007) GABAergic inhibition at dendrodendritic synapses tunes gamma oscillations in the olfactory bulb. Proceedings of the National Academy of Sciences of the United States of America 104:7259-7264.

Lardi-Studler B, Smolinsky B, Petitjean CM, Koenig F, Sidler C, Meier JC, Fritschy JM, Schwarz G (2007) Vertebrate-specific sequences in the gephyrin E-domain regulate cytosolic aggregation and postsynaptic clustering. Journal of cell science 120:1371-1382.

Ledergerber D, Fritschy JM, Kralic JE (2006) Impairment of dentate gyrus neuronal progenitor cell differentiation in a mouse model of temporal lobe epilepsy. Experimental neurology 199:130-142.

Lepousez G, Lledo PM (2013) Odor discrimination requires proper olfactory fast oscillations in awake mice. Neuron 80:1010-1024.

Lepousez G, Valley MT, Lledo PM (2013) The impact of adult neurogenesis on olfactory bulb circuits and computations. Annual review of physiology 75:339-363.

Levi S, Logan SM, Tovar KR, Craig AM (2004) Gephyrin is critical for glycine receptor clustering but not for the formation of functional GABAergic synapses in hippocampal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:207-217.

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nature reviews Neuroscience 6:312-324.

Li BS, Ma W, Jaffe H, Zheng Y, Takahashi S, Zhang L, Kulkarni AB, Pant HC (2003) Cyclin-dependent kinase-5 is involved in neuregulin-dependent activation of phosphatidylinositol 3-kinase and Akt activity mediating neuronal survival. The Journal of biological chemistry 278:35702-35709.

Lieberwirth C, Liu Y, Jia X, Wang Z (2012) Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. Hormones and behavior 62:357-366.

Liu MT, Wuebbens MM, Rajagopalan KV, Schindelin H (2000) Crystal structure of the gephyrin-related molybdenum cofactor biosynthesis protein MogA from Escherichia coli. The Journal of biological chemistry 275:1814-1822.

Liu X, Wang Q, Haydar TF, Bordey A (2005) Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. Nature neuroscience 8:1179-1187.

Livneh Y, Mizrahi A (2012) Experience-dependent plasticity of mature adult-born neurons. Nature neuroscience 15:26-28.

Lledo PM, Alonso M, Grubb MS (2006) Adult neurogenesis and functional plasticity in neuronal circuits. Nature reviews Neuroscience 7:179-193.

Loebrich S, Bahring R, Katsuno T, Tsukita S, Kneussel M (2006) Activated radixin is essential for GABAA receptor alpha5 subunit anchoring at the actin cytoskeleton. The EMBO journal 25:987-999.

Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. Science 264:1145-1148.

Lois C, Garcia-Verdugo JM, Alvarez-Buylla A (1996) Chain migration of neuronal precursors. Science 271:978-981.

Luscher B, Fuchs T, Kilpatrick CL (2011a) GABAA receptor trafficking-mediated plasticity of inhibitory synapses. Neuron 70:385-409.

Luscher B, Shen Q, Sahir N (2011b) The GABAergic deficit hypothesis of major depressive disorder. Molecular psychiatry 16:383-406.

Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173-189.

Ma DK, Kim WR, Ming GL, Song H (2009) Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. Annals of the New York Academy of Sciences 1170:664-673.

Ma M (2007) Encoding olfactory signals via multiple chemosensory systems. Critical reviews in biochemistry and molecular biology 42:463-480.

Maas C, Belgardt D, Lee HK, Heisler FF, Lappe-Siefke C, Magiera MM, van Dijk J, Hausrat TJ, Janke C, Kneussel M (2009) Synaptic activation modifies microtubules underlying transport of postsynaptic cargo. Proceedings of the National Academy of Sciences of the United States of America 106:8731-8736.

Macdonald RL, Olsen RW (1994) GABAA receptor channels. Annual review of neuroscience 17:569-602.

Magill ST, Cambronne XA, Luikart BW, Lioy DT, Leighton BH, Westbrook GL, Mandel G, Goodman RH (2010) microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. Proceedings of the National Academy of Sciences of the United States of America 107:20382-20387.

Mammoto A, Sasaki T, Asakura T, Hotta I, Imamura H, Takahashi K, Matsuura Y, Shirao T, Takai Y (1998) Interactions of drebrin and gephyrin with profilin. Biochemical and biophysical research communications 243:86-89.

Maric HM, Mukherjee J, Tretter V, Moss SJ, Schindelin H (2011) Gephyrin-mediated gamma-aminobutyric acid type A and glycine receptor clustering relies on a common binding site. The Journal of biological chemistry 286:42105-42114.

Marin-Burgin A, Mongiat LA, Pardi MB, Schinder AF (2012) Unique processing during a period of high excitation/inhibition balance in adult-born neurons. Science 335:1238-1242.

Markwardt SJ, Dieni CV, Wadiche JI, Overstreet-Wadiche L (2011) Ivy/neurogliaform interneurons coordinate activity in the neurogenic niche. Nature neuroscience 14:1407-1409.

Marsden KC, Beattie JB, Friedenthal J, Carroll RC (2007) NMDA receptor activation potentiates inhibitory transmission through GABA receptor-associated protein-dependent exocytosis of GABA(A) receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:14326-14337.

Marsden KC, Shemesh A, Bayer KU, Carroll RC (2010) Selective translocation of Ca2+/calmodulin protein kinase IIalpha (CaMKIIalpha) to inhibitory synapses. Proceedings of the National Academy of Sciences of the United States of America 107:20559-20564.

Mayer S, Kumar R, Jaiswal M, Soykan T, Ahmadian MR, Brose N, Betz H, Rhee JS, Papadopoulos T (2013) Collybistin activation by GTP-TC10 enhances postsynaptic gephyrin clustering and hippocampal GABAergic neurotransmission. Proceedings of the National Academy of Sciences of the United States of America 110:20795-20800.

Mejia-Gervacio S, Murray K, Lledo PM (2011) NKCC1 controls GABAergic signaling and neuroblast migration in the postnatal forebrain. Neural development 6:4.

Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annual review of neuroscience 28:223-250.

Ming GL, Song H (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70:687-702.

Mirochnic S, Wolf S, Staufenbiel M, Kempermann G (2009) Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. Hippocampus 19:1008-1018.

Mitchell EA, Herd MB, Gunn BG, Lambert JJ, Belelli D (2008) Neurosteroid modulation of GABAA receptors: molecular determinants and significance in health and disease. Neurochemistry international 52:588-595.

Miyamichi K, Amat F, Moussavi F, Wang C, Wickersham I, Wall NR, Taniguchi H, Tasic B, Huang ZJ, He Z, Callaway EM, Horowitz MA, Luo L (2011) Cortical representations of olfactory input by trans-synaptic tracing. Nature 472:191-196.

Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R (1996) Visualizing an olfactory sensory map. Cell 87:675-686.

Mori K, Nagao H, Yoshihara Y (1999) The olfactory bulb: coding and processing of odor molecule information. Science 286:711-715.

Mouret A, Lepousez G, Gras J, Gabellec MM, Lledo PM (2009) Turnover of newborn olfactory bulb neurons optimizes olfaction. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:12302-12314.

Murk K, Wittenmayer N, Michaelsen-Preusse K, Dresbach T, Schoenenberger CA, Korte M, Jockusch BM, Rothkegel M (2012) Neuronal profilin isoforms are addressed by different signalling pathways. PloS one 7:e34167.

Ng T, Ryu JR, Sohn JH, Tan T, Song H, Ming GL, Goh EL (2013) Class 3 semaphorin mediates dendrite growth in adult newborn neurons through Cdk5/FAK pathway. PloS one 8:e65572.

Nikolic M, Chou MM, Lu W, Mayer BJ, Tsai LH (1998) The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. Nature 395:194-198.

Ninkovic J, Mori T, Gotz M (2007) Distinct modes of neuron addition in adult mouse neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:10906-10911.

Nissant A, Bardy C, Katagiri H, Murray K, Lledo PM (2009) Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. Nature neuroscience 12:728-730.

Nissant A, Pallotto M (2011) Integration and maturation of newborn neurons in the adult olfactory bulb--from synapses to function. The European journal of neuroscience 33:1069-1077.

Notter T, Panzanelli P, Pfister S, Mircsof D, Fritschy JM (2014) A protocol for concurrent high-quality immunohistochemical and biochemical analyses in adult mouse central nervous system. The European journal of neuroscience 39:165-175.

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:1693-1703.

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain research 34:171-175.

Obrietan K, Gao XB, Van Den Pol AN (2002) Excitatory actions of GABA increase BDNF expression via a MAPK-CREB-dependent mechanism--a positive feedback circuit in developing neurons. Journal of neurophysiology 88:1005-1015.

Olsen RW, Sieghart W (2009) GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141-148.

Pallotto M, Deprez F (2014) Regulation of adult neurogenesis by GABAergic transmission: signaling beyond GABAA-receptors. Frontiers in cellular neuroscience 8:166.

Pallotto M, Nissant A, Fritschy JM, Rudolph U, Sassoe-Pognetto M, Panzanelli P, Lledo PM (2012) Early formation of GABAergic synapses governs the development of adult-born neurons in the olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:9103-9115.

Panzanelli P, Bardy C, Nissant A, Pallotto M, Sassoe-Pognetto M, Lledo PM, Fritschy JM (2009) Early synapse formation in developing interneurons of the adult olfactory bulb. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:15039-15052.

Panzanelli P, Gunn BG, Schlatter MC, Benke D, Tyagarajan SK, Scheiffele P, Belelli D, Lambert JJ, Rudolph U, Fritschy JM (2011) Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. The Journal of physiology 589:4959-4980.

Panzanelli P, Perazzini AZ, Fritschy JM, Sassoe-Pognetto M (2005) Heterogeneity of gamma-aminobutyric acid type A receptors in mitral and tufted cells of the rat main olfactory bulb. The Journal of comparative neurology 484:121-131.

Papadopoulos T, Korte M, Eulenburg V, Kubota H, Retiounskaia M, Harvey RJ, Harvey K, O'Sullivan GA, Laube B, Hulsmann S, Geiger JR, Betz H (2007) Impaired GABAergic transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. The EMBO journal 26:3888-3899.

Papale A, Cerovic M, Brambilla R (2009) Viral vector approaches to modify gene expression in the brain. Journal of neuroscience methods 185:1-14.

Patrizi A, Scelfo B, Viltono L, Briatore F, Fukaya M, Watanabe M, Strata P, Varoqueaux F, Brose N, Fritschy JM, Sassoe-Pognetto M (2008) Synapse formation and clustering of neuroligin-2 in the absence of GABAA receptors. Proceedings of the National Academy of Sciences of the United States of America 105:13151-13156.

Paysan J, Kossel A, Bolz J, Fritschy JM (1997) Area-specific regulation of gamma-aminobutyric acid type A receptor subtypes by thalamic afferents in developing rat neocortex. Proceedings of the National Academy of Sciences of the United States of America 94:6995-7000.

Petreanu L, Alvarez-Buylla A (2002) Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:6106-6113.

Pfeiffer F, Graham D, Betz H (1982) Purification by affinity chromatography of the glycine receptor of rat spinal cord. The Journal of biological chemistry 257:9389-9393.

Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Baron-Van Evercooren A (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proceedings of the National Academy of Sciences of the United States of America 99:13211-13216.

Platel JC, Lacar B, Bordey A (2007) GABA and glutamate signaling: homeostatic control of adult forebrain neurogenesis. Journal of molecular histology 38:303-311.

Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzacher SW, Betz H, Harvey RJ, Brose N, Zhang W, Varoqueaux F (2009) Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 63:628-642.

Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE (2006) Specific subtypes of GABAA receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. Journal of neurophysiology 96:846-857.

Price JL, Powell TP (1970) The synaptology of the granule cells of the olfactory bulb. Journal of cell science 7:125-155.

Prut L, Prenosil G, Willadt S, Vogt K, Fritschy JM, Crestani F (2010) A reduction in hippocampal GABAA receptor alpha5 subunits disrupts the memory for location of objects in mice. Genes, brain, and behavior 9:478-488.

Rakic P (2002) Adult neurogenesis in mammals: an identity crisis. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:614-618.

Ramos B, Lopez-Tellez JF, Vela J, Baglietto-Vargas D, del Rio JC, Ruano D, Gutierrez A, Vitorica J (2004) Expression of alpha 5 GABAA receptor subunit in developing rat hippocampus. Brain research Developmental brain research 151:87-98.

Ren Z, Sahir N, Murakami S, Luellen BA, Earnheart JC, Lal R, Kim JY, Song H, Luscher B (2014) Defects in dendrite and spine maturation and synaptogenesis associated with an anxious-depressive-like phenotype of GABA receptor-deficient mice. Neuropharmacology.

Represa A, Ben-Ari Y (2005) Trophic actions of GABA on neuronal development. Trends in neurosciences 28:278-283.

Ressler KJ, Sullivan SL, Buck LB (1994) Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell 79:1245-1255.

Rudolph U, Mohler H (2004) Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annual review of pharmacology and toxicology 44:475-498.

Rudolph U, Mohler H (2014) GABAA receptor subtypes: Therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annual review of pharmacology and toxicology 54:483-507.

Rui Y, Myers KR, Yu K, Wise A, De Blas AL, Hartzell HC, Zheng JQ (2013) Activity-dependent regulation of dendritic growth and maintenance by glycogen synthase kinase 3beta. Nature communications 4:2628.

Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton AA, Dranovsky A, Hen R (2011) Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472:466-470.

Saiepour L, Fuchs C, Patrizi A, Sassoe-Pognetto M, Harvey RJ, Harvey K (2010) Complex role of collybistin and gephyrin in GABAA receptor clustering. The Journal of biological chemistry 285:29623-29631.

Sassoe-Pognetto M, Kirsch J, Grunert U, Greferath U, Fritschy JM, Mohler H, Betz H, Wassle H (1995) Colocalization of gephyrin and GABAA-receptor subunits in the rat retina. The Journal of comparative neurology 357:1-14.

Sassoe-Pognetto M, Panzanelli P, Sieghart W, Fritschy JM (2000) Colocalization of multiple GABA(A) receptor subtypes with gephyrin at postsynaptic sites. The Journal of comparative neurology 420:481-498.

Scharfman HE, Hen R (2007) Neuroscience. Is more neurogenesis always better? Science 315:336-338.

Scheiffele P, Fan J, Choih J, Fetter R, Serafini T (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 101:657-669.

Schlomann U, Schwamborn JC, Muller M, Fassler R, Puschel AW (2009) The stimulation of dendrite growth by Sema3A requires integrin engagement and focal adhesion kinase. Journal of cell science 122:2034-2042.

Schwarz G, Mendel RR, Ribbe MW (2009) Molybdenum cofactors, enzymes and pathways. Nature 460:839-847.

Schwer B, Eckersdorff M, Li Y, Silva JC, Fermin D, Kurtev MV, Giallourakis C, Comb MJ, Alt FW, Lombard DB (2009) Calorie restriction alters mitochondrial protein acetylation. Aging cell 8:604-606.

Scott JW, McBride RL, Schneider SP (1980) The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. The Journal of comparative neurology 194:519-534.

Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:7153-7160.

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of GABAA receptors containing the alpha5 subunit in the rat brain. The Journal of comparative neurology 499:458-470.

Shen Q, Fuchs T, Sahir N, Luscher B (2012) GABAergic control of critical developmental periods for anxiety- and depression-related behavior in mice. PloS one 7:e47441.

Shepherd GM, Chen WR, Willhite D, Migliore M, Greer CA (2007) The olfactory granule cell: from classical enigma to central role in olfactory processing. Brain research reviews 55:373-382.

Shimojima K, Sugawara M, Shichiji M, Mukaida S, Takayama R, Imai K, Yamamoto T (2011) Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. Journal of human genetics 56:561-565.

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, Adamiker D (1999) Structure and subunit composition of GABA(A) receptors. Neurochemistry international 34:379-385.

Sieghart W, Sperk G (2002) Subunit composition, distribution and function of GABA(A) receptor subtypes. Current topics in medicinal chemistry 2:795-816.

Smith KR, Davenport EC, Wei J, Li X, Pathania M, Vaccaro V, Yan Z, Kittler JT (2014) GIT1 and betaPIX Are Essential for GABAA Receptor Synaptic Stability and Inhibitory Neurotransmission. Cell reports 9:298-310.

Sola M, Bavro VN, Timmins J, Franz T, Ricard-Blum S, Schoehn G, Ruigrok RW, Paarmann I, Saiyed T, O'Sullivan GA, Schmitt B, Betz H, Weissenhorn W (2004) Structural basis of dynamic glycine receptor clustering by gephyrin. The EMBO journal 23:2510-2519.

Sola M, Kneussel M, Heck IS, Betz H, Weissenhorn W (2001) X-ray crystal structure of the trimeric N-terminal domain of gephyrin. The Journal of biological chemistry 276:25294-25301.

Song J, Christian KM, Ming GL, Song H (2012a) Modification of hippocampal circuitry by adult neurogenesis. Developmental neurobiology 72:1032-1043.

Song J, Zhong C, Bonaguidi MA, Sun GJ, Hsu D, Gu Y, Meletis K, Huang ZJ, Ge S, Enikolopov G, Deisseroth K, Luscher B, Christian KM, Ming GL, Song H (2012b) Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature 489:150-154.

Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Bostrom E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisen J (2013) Dynamics of hippocampal neurogenesis in adult humans. Cell 153:1219-1227.

Squire LR (1992) Declarative and nondeclarative memory: multiple brain systems supporting learning and memory. Journal of cognitive neuroscience 4:232-243.

Stewart RR, Hoge GJ, Zigova T, Luskin MB (2002) Neural progenitor cells of the neonatal rat anterior subventricular zone express functional GABA(A) receptors. Journal of neurobiology 50:305-322.

Studler B, Sidler C, Fritschy JM (2005) Differential regulation of GABA(A) receptor and gephyrin postsynaptic clustering in immature hippocampal neuronal cultures. The Journal of comparative neurology 484:344-355.

Sudhof TC (2008) Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455:903-911.

Sun J, Ming GL, Song H (2011) Epigenetic regulation of neurogenesis in the adult mammalian brain. The European journal of neuroscience 33:1087-1093.

Tan KR, Rudolph U, Luscher C (2011) Hooked on benzodiazepines: GABAA receptor subtypes and addiction. Trends in neurosciences 34:188-197.

Thomas P, Mortensen M, Hosie AM, Smart TG (2005) Dynamic mobility of functional GABAA receptors at inhibitory synapses. Nature neuroscience 8:889-897.

Timofeeva OA, Levin ED (2011) Glutamate and nicotinic receptor interactions in working memory: importance for the cognitive impairment of schizophrenia. Neuroscience 195:21-36.

Toni N, Sultan S (2011) Synapse formation on adult-born hippocampal neurons. The European journal of neuroscience 33:1062-1068.

Tretter V, Mukherjee J, Maric HM, Schindelin H, Sieghart W, Moss SJ (2012) Gephyrin, the enigmatic organizer at GABAergic synapses. Frontiers in cellular neuroscience 6:23.

Tronel S, Belnoue L, Grosjean N, Revest JM, Piazza PV, Koehl M, Abrous DN (2012) Adult-born neurons are necessary for extended contextual discrimination. Hippocampus 22:292-298.

Tyagarajan SK, Fritschy JM (2014) Gephyrin: a master regulator of neuronal function? Nature reviews Neuroscience 15:141-156.

Tyagarajan SK, Ghosh H, Harvey K, Fritschy JM (2011a) Collybistin splice variants differentially interact with gephyrin and Cdc42 to regulate gephyrin clustering at GABAergic synapses. Journal of cell science 124:2786-2796.

Tyagarajan SK, Ghosh H, Yevenes GE, Imanishi SY, Zeilhofer HU, Gerrits B, Fritschy JM (2013) Extracellular signal-regulated kinase and glycogen synthase kinase 3beta regulate gephyrin postsynaptic aggregation and GABAergic synaptic function in a calpain-dependent mechanism. The Journal of biological chemistry 288:9634-9647.

Tyagarajan SK, Ghosh H, Yevenes GE, Nikonenko I, Ebeling C, Schwerdel C, Sidler C, Zeilhofer HU, Gerrits B, Muller D, Fritschy JM (2011b) Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. Proceedings of the National Academy of Sciences of the United States of America 108:379-384.

Vadodaria KC, Brakebusch C, Suter U, Jessberger S (2013) Stage-specific functions of the small Rho GTPases Cdc42 and Rac1 for adult hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:1179-1189.

Vadodaria KC, Jessberger S (2013) Maturation and integration of adult born hippocampal neurons: signal convergence onto small Rho GTPases. Frontiers in synaptic neuroscience 5:4.

van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. Nature reviews Neuroscience 1:191-198.

van Rijnsoever C, Tauber M, Choulli MK, Keist R, Rudolph U, Mohler H, Fritschy JM, Crestani F (2004) Requirement of alpha5-GABAA receptors for the development of tolerance to the sedative action of diazepam in mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:6785-6790.

Varela F, Lachaux JP, Rodriguez E, Martinerie J (2001) The brainweb: phase synchronization and large-scale integration. Nature reviews Neuroscience 2:229-239.

Vargas-Caballero M, Martin LJ, Salter MW, Orser BA, Paulsen O (2010) alpha5 Subunit-containing GABA(A) receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation. Neuropharmacology 58:668-675.

Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Sudhof TC, Brose N (2006) Neuroligins determine synapse maturation and function. Neuron 51:741-754.

Varoqueaux F, Jamain S, Brose N (2004) Neuroligin 2 is exclusively localized to inhibitory synapses. European journal of cell biology 83:449-456.

Wang DD, Kriegstein AR (2009) Defining the role of GABA in cortical development. The Journal of physiology 587:1873-1879.

Wang DD, Krueger DD, Bordey A (2003) GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAA receptor activation. The Journal of physiology 550:785-800.

Whitman MC, Greer CA (2007) Synaptic integration of adult-generated olfactory bulb granule cells: basal axodendritic centrifugal input precedes apical dendrodendritic local circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:9951-9961.

Whitman MC, Greer CA (2009) Adult neurogenesis and the olfactory system. Progress in neurobiology 89:162-175.

Wilson SW, Rubenstein JL (2000) Induction and dorsoventral patterning of the telencephalon. Neuron 28:641-651.

Winner B, Kohl Z, Gage FH (2011) Neurodegenerative disease and adult neurogenesis. The European journal of neuroscience 33:1139-1151.

Wolf SA, Melnik A, Kempermann G (2011) Physical exercise increases adult neurogenesis and telomerase activity, and improves behavioral deficits in a mouse model of schizophrenia. Brain, behavior, and immunity 25:971-980.

Xiang S, Kim EY, Connelly JJ, Nassar N, Kirsch J, Winking J, Schwarz G, Schindelin H (2006) The crystal structure of Cdc42 in complex with collybistin II, a gephyrin-interacting guanine nucleotide exchange factor. Journal of molecular biology 359:35-46.

Xing Y, Sonner J, Laster MJ, Abaigar W, Caraiscos VB, Orser B, Eger EI, 2nd (2004) Insulin decreases isoflurane minimum alveolar anesthetic concentration in rats independently of an effect on the spinal cord. Anesthesia and analgesia 98:1712-1717, table of contents.

Yamaguchi M, Mori K (2005) Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. Proceedings of the National Academy of Sciences of the United States of America 102:9697-9702.

Yau SY, Lau BW, So KF (2011) Adult hippocampal neurogenesis: a possible way how physical exercise counteracts stress. Cell transplantation 20:99-111.

Yee BK, Hauser J, Dolgov VV, Keist R, Mohler H, Rudolph U, Feldon J (2004) GABA receptors containing the alpha5 subunit mediate the trace effect in aversive and appetitive conditioning and extinction of conditioned fear. The European journal of neuroscience 20:1928-1936.

Yokoi M, Mori K, Nakanishi S (1995) Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. Proceedings of the National Academy of Sciences of the United States of America 92:3371-3375.

Yu W, Jiang M, Miralles CP, Li RW, Chen G, de Blas AL (2007) Gephyrin clustering is required for the stability of GABAergic synapses. Molecular and cellular neurosciences 36:484-500.

Zald DH, Pardo JV (2000) Functional neuroimaging of the olfactory system in humans. International journal of psychophysiology : official journal of the International Organization of Psychophysiology 36:165-181.

Zarnowska ED, Keist R, Rudolph U, Pearce RA (2009) GABAA receptor alpha5 subunits contribute to GABAA,slow synaptic inhibition in mouse hippocampus. Journal of neurophysiology 101:1179-1191.

Zhang H, Webb DJ, Asmussen H, Niu S, Horwitz AF (2005) A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:3379-3388.

Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132:645-660.

Zhao C, Teng EM, Summers RG, Jr., Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:3-11.

Zhao S, Zhou Y, Gross J, Miao P, Qiu L, Wang D, Chen Q, Feng G (2010) Fluorescent labeling of newborn dentate granule cells in GAD67-GFP transgenic mice: a genetic tool for the study of adult neurogenesis. PloS one 5.

ABBREVIATIONS

ACSF	Artificial cerebrospinal fluid
BDNF	Brain-derived neurotrophic factor
BZ	Benzodiazepine
CaMK	Calmodulin-dependent protein kinase
CB	Collybistin
Cdk	Cycline-dependent kinase
CNS	Central nervous system
CREB	cAMP response element-binding protein
DG	Dentate Gyrus
dpi	Days post-injection
EC	Entorhinal Cortex
EGF	Epidermal growth factor
E/I	Excitation and inhibition
eIPSC	evoked inhibitory postsynaptic current
EPL	External plexiform layer
ERK	Extracellular regulated kinase
FAK	Focal adhesion kinase
GABA	γ-aminobutyric acid
$GABA_AR$	$GABA_A$ Receptor
GC	Granule cell
GCL	Granule cell layer
GEF	Guanine nucleotide exchange factor
eGFP	Enhanced Green fluorescent protein
GL	Glomerular layer
GlyR	Glycine receptor
GSK3β	Glycogen synthase kinase 3β
Het	Heterozygous
i.p.	Intraperitoneal injection
IPL	Internal plexiform layer
KCC2	K+-Cl- co-transporter
KO	Knockout

LTD	Long-term depression
LTP	Long-term potentiation
LV	Lentivirus
MC	Mitral cell
MCL	Mitral cell layer
mIPSC	miniature inhibitory postsynaptic current
MOCO	Molybdenum cofactor
mRFP	monomeric red fluorescent protein
NCAM	Neural cell adhesion molecule
NGF	Neural growth factor
NGS	Normal goat serum
NKCC1	Na+-K+-2Cl- co-transporter
NL	Neuroligin
NPC	Neural progenitor cell
NSC	Neural stem cell
OB	Olfactory bulb
OSN	Olfactory sensory neuron
PAX6	Paired box 6
PBS	Phosphate-buffered saline
PGC	Periglomerular cell
PKA	Protein kinase A
PSA-NCAM	polysialylated-neural cell adhesion molecule
PSD	Postsynaptic density
PV^+	Parvalbumin positive
Rac	Ras-related C3
RMS	Rostral migratory stream
ROD	Relative optical density
RV	Retrovirus
SGZ	Subgranular zone
TC	Tufted cell
TM	Transmembrane
SVZ	Subventricular zone
VEGF	Vascular endothelial growth factor
vGAT	vesicular GABA transporter

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Zattoni M, Mura ML, Deprez F, Schwendener RA, Engelhardt B, Frei K, Fritschy JM. *Brain infiltration of leukocytes contributes to the pathophysiology of temporal lobe epilepsy*. J Neurosci. 2011 Mar;31(11):4037-50.

Pallotto M, Deprez F. *Regulation of adult neurogenesis by GABAergic transmission: signaling beyond GABAA-receptors*. Front Cell Neurosci. 2014 Jun;8:166.

Abstracts for Posters

F. Deprez, M. Pallotto, M. Grabiec, SK. Tyagarajan, P. Panzanelli, P.-M. Lledo, J.-M. Fritschy (2012). *Formation and regulation of GABAergic synapses and role of gephyrin in adult neurogenesis in the olfactory bulb*. NCCR Neuro Concluding Symposium and ZNZ Annual Symposium, Neural Plasticity and Repair, from basic Neuroscience to Therapy, Zurich, Switzerland

F. Deprez, M. Grabiec, M. Pallotto, V. Duveau, SK. Tyagarajan, P. Panzanelli, J.-M. Fritschy (2012). *Role of gephyrin for maturation of newborn neurons in the olfactory bulb*. 8th Forum of European Neuroscience (FENS), Barcelona, Spain

F. Deprez, M. Pallotto, V. Duveau, SK. Tyagarajan, P. Panzanelli, J.-M. Fritschy (2013). *Role of gephyrin for maturation of newborn neurons in the olfactory bulb*. Annual Gordon Research Conferences (GRC), Les Diablerets, Switzerland

F. Deprez, M. Pallotto, V. Duveau, SK. Tyagarajan, P. Panzanelli, J.-M. Fritschy (2013). *Role of gephyrin for maturation of newborn neurons in the olfactory bulb*. Annual Meeting of the Society of Neuroscience (SfN), San Diego, CA, USA

F. Deprez, M. Pallotto, V. Duveau, SK. Tyagarajan, P. Panzanelli, J.-M. Fritschy (2013). *Role of gephyrin for maturation of newborn neurons in the olfactory bulb.* Mechanisms of GABAergic synaptic plasticity, Chexbres, Switzerland

Invited Talks

Annual Pharmacology and Toxicology Poster Day (Zürich, Switzerland), August 2013: "*Synaptic integration and neuronal maturation of granule cells during adult neurogenesis in the olfactory bulb"*

APPENDIX

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
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Regulation of adult neurogenesis by GABAergic transmission: signaling beyond $GABA_A$-receptors

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In the adult mammalian brain, neurogenesis occurs in the olfactory bulb (OB) and in the dentate gyrus (DG) of the hippocampus. Several studies have shown that multiple stages of neurogenesis are regulated by GABAergic transmission with precise spatio-temporal selectivity, and involving mechanisms common to both systems or specific only to one. In the subgranular zone (SGZ) of the DG, GABA neurotransmitter, released by a specific population of interneurons, regulates stem cell quiescence and neuronal cell fate decisions. Similarly, in the subventricular zone (SVZ), OB neuroblast production is modulated by ambient GABA. Ambient GABA, acting on extrasynaptic $GABA_A$ receptors ($GABA_AR$), is also crucial for proper adult-born granule cell (GC) maturation and synaptic integration in the OB as well as in the DG. Throughout adult-born neuron development, various GABA receptors and receptor subunits play specific roles. Previous work has demonstrated that adult-born GCs in both the OB and the DG show a time window of increased plasticity in which adult-born cells are more prone to modification by external stimuli. One mechanism that controls this "critical period" is GABAergic modulation. Indeed, depleting the main phasic GABAergic inputs in adult-born neurons results in dramatic effects, such as reduction of spine density and dendritic branching in adult-born OB GCs. In this review, we systematically compare the role of GABAergic transmission in the regulation of adult neurogenesis between the OB and the hippocampus, focusing on the role of GABA in modulating plasticity and critical periods of adult-born neuron development. Finally, we discuss signaling pathways that might mediate some of the deficits observed upon targeted deletion of postsynaptic $GABA_A$Rs in adult-born neurons.

Keywords: adult neurogenesis, olfactory bulb, dentate gyrus, $GABA_A$ receptor, plasticity

ADULT NEUROGENESIS AND GABAergic SIGNALING

Brain development depends on the coordination of numerous processes that go from cell proliferation to circuit refinement. In mature brain circuits, γ-aminobutyric acid (GABA) acts as the main inhibitory neurotransmitter. It is now well known that GABA plays more than a classical inhibitory role and can function as an important developmental signal early in life. Its actions influence processes such as proliferation of neuroblasts and migration, synapse formation, and synapse plasticity. Therefore, GABAergic transmission is essential for proper brain formation and functioning. Imbalance between excitation and inhibition (E/I) due to impaired GABAergic signaling has been implicated in several diseases, such as schizophrenia, epilepsy, autism-spectrum disorders, and intellectual disability. Similarly, GABA exerts a fundamental role in regulating adult neurogenesis, which allows its effects on developing neurons to be studied in adult tissue. The role of GABAergic signaling has been long studied (Bovetti et al., 2011; Berg et al., 2013). Here we will focus on the role of $GABA_AR$ subunits plays in adult neurogenesis.

In the first part of this review, we will briefly describe the crucial phases that lead a neural stem cell to differentiate and become an adult-born neuron in the dentate gyrus (DG) of the hippocampus and in the olfactory bulb (OB). Then, we will describe the molecular organization of $GABA_A$ receptors ($GABA_AR$). Finally, we will illustrate the role of GABAergic signals regulating adult neurogenesis. Our goal is to discuss how the spatio-temporal regulation of GABAergic transmission through distinct $GABA_AR$ subtypes is involved in modulating adult neurogenesis in the OB and DG. In doing so, we will compare the two systems in order to identify common and unique mechanisms mediated by GABAergic transmission.

NEUROGENESIS IN THE ADULT BRAIN

Adult neurogenesis is the life-long continuous production and functional integration of newborn neurons in the CNS. It represents a process by which the brain can modify itself to face and adapt to external stimuli, as well as to learn and remember. In the rodent brain, adult neurogenesis is restricted to two specific neurogenic niches, the subgranular zone (SGZ) of the DG and the subventricular zone (SVZ) of the lateral ventricles (Gage, 2000; Alvarez-Buylla et al., 2001; Rakic, 2002). The steps of adult neurogenesis include proliferation of stem and progenitor cells, neuroblast fate specification and migration, neuronal differentiation, survival, and integration into the existing circuitry. These

steps are under precise spatial and temporal control, but can be modulated by both internal and external stimuli.

In the SVZ three different types of neural precursor cells have been identified: type B radial glia-like progenitors, type C transient amplifying cells and type A migrating neuroblasts. Type B cells divide slowly and give rise to transient amplifying cells and oligodendrocytes. Type C cells divide rapidly and give rise to migrating neuroblasts (Abrous et al., 2005). Neuroblasts exhibit an elongated cell body and have two radially opposed processes (Lois and Alvarez-Buylla, 1993). Before differentiating into olfactory GCs and periglomerular cells (PGCs), these neuroblasts have to migrate for a long distance through the rostral migratory stream (RMS) toward the OB (**Figure 1B**). During migration, they form a chain consisting of a group of 30–40 cells. After about 5 days, neuroblasts reach the OB, detach from the chain in the RMS and start to migrate to reach either the granule cell layer (GCL) or the glomerular layer (GL) (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois et al., 1996). Here they form distinct populations of interneurons, mainly located in the GCL (50–75%) and to a lesser extent in the GL (25%) (Luskin, 1993). Their integration into the preexisting circuitry occurs rapidly. One or two days after entry in the GCL, OB-GCs first receive axo-dendritic inputs from local interneurons (short axon cells), mitral cells, tufted cells, and centrifugal fibers. Finally, upon entering the external plexiform layer (EPL), the dendrite starts branching and forms dendro-dendritic reciprocal contacts with mitral and tufted cells (Whitman and Greer, 2007; Panzanelli et al., 2009). PGCs rapidly migrate toward the GL, send their axon into one or several glomeruli and receive inputs from the olfactory sensory nerve and dendro-dendritic contacts from mitral and tufted cells (Shao et al., 2009). While OB-GCs are strictly GABAergic, PGC can express various transmitter phenotypes including GABAergic, dopaminergic and glutamatergic, depending on their site of origin in the SGZ/RMS (**Figure 1C**).

The SGZ of the DG contains radial glia-like quiescent neural stem cells (Type I cells), that undergo symmetric division, or give rise to intermediate progenitor cells (Type II), astrocytes or oligodendrocytes. Postmitotic intermediate progenitors differentiate as neuroblasts, which migrate a short distance into the inner GCL and differentiate as GCs. Dentate GCs receive their main excitatory input from the enthorinal cortex and provide glutamatergic inputs to hippocampal pyramidal neurons and CA3 inhibitory interneurons (**Figure 1A**).

MOLECULAR ORGANIZATION OF $GABA_A$ RECEPTORS

Among the numerous factors regulating adult neurogenesis, GABAergic signaling, primarily through $GABA_A$Rs, plays a major role. $GABA_A$Rs are ligand-gated Cl^- channels mediating most of the fast inhibitory action of GABA. $GABA_A$Rs are also permeable to HCO^{3-} (Kaila et al., 1992), which decrease the effect of inhibition of the Cl^- entry, leading to depolarization. $GABA_A$Rs are encoded by a large family of subunit genes, grouped in seven classes according to their sequence homology: α (1–6), β (1–3), γ (1–3), δ, ε, θ, π and ρ (1–3) (Macdonald and Olsen, 1994; Sieghart et al., 1999; Sieghart and Sperk, 2002). Differential assembly of these subunits in pentameric channels results in multiple $GABA_A$R subtypes with unique functional and pharmacological properties [for α1 subunit, (Kralic et al., 2006), for α3 (Studler et al., 2005), for α5 (Fritschy et al., 1997)].

$GABA_A$R subtypes mediating synaptic GABAergic transmission in mature neurons are composed of two α1, α2, α3 subunits together with two β2 or β3 and one γ2 subunit (Jacob et al., 2008). In contrast, the receptors composed of α4, α5, or α6 subunit variants, along with β subunits and δ or γ2 (located at the extrasynaptic sites) mediate tonic GABAergic transmission through ambient GABA (Kilb et al., 2013).

Receptor properties, such as trafficking or clustering, can be regulated by interactions with scaffold proteins and major signaling complexes. Gephyrin, a phospho-protein, is the main postsynaptic scaffolding protein both for GABAergic and glycinergic synapses. It is essential for stabilization of $GABA_A$Rs but also interacts with other postsynaptic proteins, like neuroligins and collybistin (Saiepour et al., 2010; Fritschy et al., 2012). It has been shown that the absence of gephyrin results in the loss of postsynaptic $GABA_A$Rs (Essrich et al., 1998; Kneussel et al., 1999). In contrast, knockout mice lacking the α1, α2 or γ2 subunits exhibit loss of gephyrin clusters (Essrich et al., 1998; Kralic et al., 2006; Patrizi et al., 2008; Panzanelli et al., 2011). *In vitro* data demonstrate that the phosphorylation state of gephyrin affects GABAergic synaptic function by regulating cluster size and density (Tyagarajan et al., 2011, 2013). Thus, abolishing the phosphorylation of residue S270 favors the formation of supernumerary synapses in cultured hippocampal neurons (Tyagarajan et al., 2011). However, it is also reported by Levi et al. (2004) that gephyrin is not strictly required for $GABA_A$R assembly, suggesting the possibility of a gephyrin- independent mechanism of inhibitory synapse development.

Collybistin, another protein which might influence the dynamic and plasticity of $GABA_A$Rs at the surface, was shown to bind gephyrin and Cdc-42, potentially affecting the remodeling of the actin cytoskeleton. Further, collybistin can bind directly to neuroligin 2, suggesting that it plays a role in maintenance of $GABA_A$R at the plasma membrane (Poulopoulos et al., 2009; Fritschy et al., 2012). These data, along with single-particle tracking studies show that the presence of synaptic and extrasynaptic $GABA_A$Rs on the plasma membrane is highly dynamic and regulated by direct or indirect interactions with postsynaptic scaffolding proteins. This feature to adapt GABAergic transmission to the differentiation of their dendrites and incoming synaptic inputs might be particularly important for developing neurons.

So far, most of the studies describing the role of different $GABA_A$R subunits are mostly based on KO mice. Despite the considerable insight into $GABA_A$R function gained from the use of KO mice, these model systems nevertheless have certain drawbacks. KO mice for $GABA_A$R subunits show compensatory effects that impact the neural circuitry, e.g., increased expression of other subunits (Kralic et al., 2006). Adult neurogenesis is moreover a process that involves maturation, integration of single cell into circuits. Given these constrains, it is important to manipulate adult generated cells independently. Recent developments have met this need with new strategies for labeling and manipulating single cells without affecting the entire circuitry. Those new strategies make use of wild type (WT) or transgenic mice model in which the injection of viral vectors in specific brain

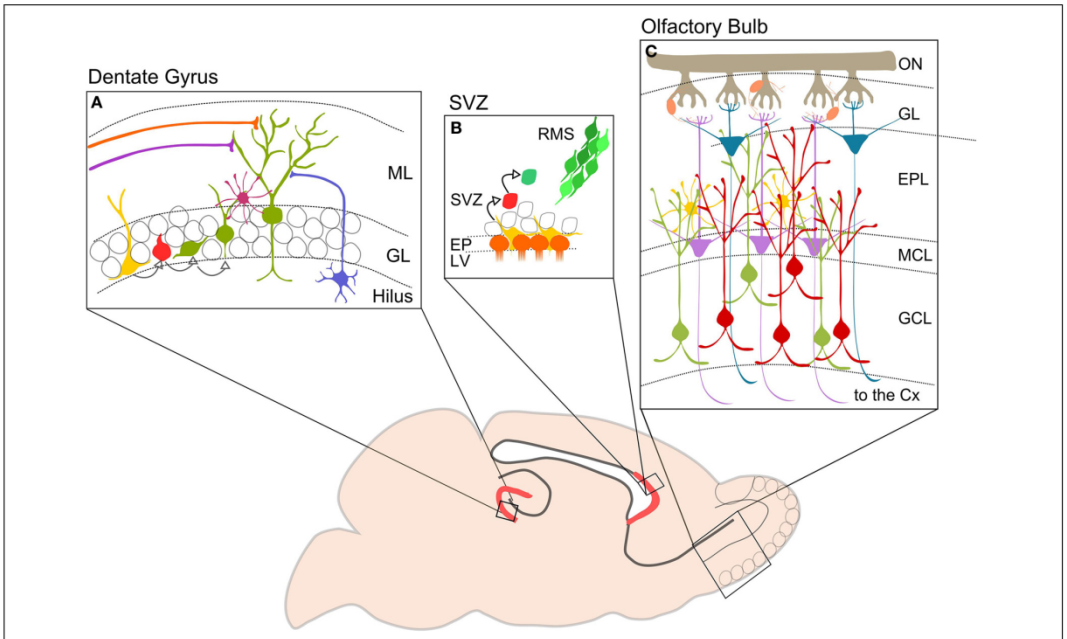

FIGURE 1 | Neurogenesis in the adult mouse brain. The picture shows in red the two neurogenerative niches: **(A)** DG of the hippocampus. Type I cells (yellow) divide to generate type II cells (red) that differentiate in immature neurons (green). DG-GCs receive GABAergic inputs from hilar interneurons (blue) and basket cells (purple), and glutamatergic inputs from lateral and medial perforant pathways (orange and violet). **(B)** SVZ. Neurogenic niche contains progenitor or type B cells (yellow) close to the ependymal cells (orange). Type b cells dived slowly and give birth to type C cells (red) which divide again in neuroblasts or type A (green). Neuroblasts migrate in chains along the RMS (from Wang and Kriegstein, 2009 modified). **(C)** Schematic representation showing OB cell types and circuitry. Axons of OSNs are collected in the ON and reach the GL where they make synapses on OB projection neurons: MCs (violet) and TCs (blue). Projection cells are modulated by interneurons, PGCs (orange) located in the GL, and perinatal and adult GCs (green and red) located in the GCL. GCs are also regulated by EPL interneurons (yellow). Axons of MCs and TCs project to higher olfactory cortical centers. ML, molecular layer; GL, granule layer; SVZ, subventricular zone; RMS, rostral migratory stream; EP, ependymal cell layer; LV, lateral ventricle; ON, olfactory nerve; ONS, olfactory sensory neuron; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granular cell layer; CX, cortex.

areas allows labeling or manipulating of specific cell population. This can be done using different promoters or, in case of adeno-associated viruses AAV, different serotype. Because of their spatial and temporal specificity, these manipulations can be done without affecting the development of the brain. This approach is particularly useful to study adult neurogenesis, and therefore is widely used in the field.

In the adult OB and DG, distinct $GABA_AR$ subtypes are expressed in various cells types to mediate both phasic and tonic inhibition, with possible functional and pharmacological specificity among distinct circuits. The subunit repertoire of precursor cells and neuroblasts is much less well established. Here, we briefly summarize what is known about their organization in both systems (**Table 1**).

In the OB, GABAergic GCs express $GABA_AR$ contain the α2 subunit, and to a lesser extent α3 subunit (Panzanelli et al., 2009), which are responsible for mediating synaptic inhibition. Immunohistochemistry also revealed the presence of extrasynaptic $GABA_ARs$ containing the α5 subunit, along with α4 and δ subunits (Panzanelli et al., 2007). GABAergic signaling in PGCs is mainly mediated through synaptic $GABA_AR$ containing the α2 subunit and extrasynaptically through the α5-$GABA_ARs$ (Panzanelli et al., 2007).

Stewart et al. have shown by RT-PCR that precursor cells and neuroblasts in the SVZ express different $GABA_ARs$ containing the α2, α3, α4, α5; β1–3 and γ2 subunits and are activated by ambient GABA release (Stewart et al., 2002). Similarly, neurospheres from striatal neuronal progenitors express α2, α4, α5; β1–3 and γ1, γ2 1νδ γ3 subunits mRNA (Nguyen et al., 2003). Therefore, while adult-born OB-GCs are regulated by both, synaptic and extrasynaptic $GABA_ARs$ differing in subunit composition, these subunits are already expressed in OB-GC precursors.

In situ hybridization (ISH) and immunohistochemical (IHC) studies have analyzed the GABAergic subunit composition. They have found that in the DG the α2- and α4-$GABA_AR$ subunits are strongly expressed, while α1 and α5 subunits are moderate expressed in the DG (Heldt and Ressler, 2007; Hortnagl et al., 2013). The $GABA_AR$ containing the α2 or α1 subunit are responsible for phasic inhibitory transmission of GCs. At extrasynaptic sites, GCs express $GABA_ARs$ with the specific subunit

Table 1 | Distribution of various $GABA_AR$ subunits in different cell types in olfactory bulb, subventricular zone (SVZ) and dentate gyrus of the hippocampus.

	Cell type	GABA R subunit	Method	References
Olfactory Bulb	Precursor cells (SVZ)	$GABA_AR$: α2, α3, α4, α5, β1-3 αvδγ 2 $GABA_AR$: α2, α4, α5; β1-3 and γ1, γ2 1vδ γ3	RT-PCR in cultured SVZ precursor cells RT-PCR from neurosphere	Stewart et al., 2002 Nguyen et al., 2003
	Granule cells	$GABA_AR$: α2, α3, α4, α5, δ subunits	Immunohistochemistry	Panzanelli et al., 2007, 2009
	Periglomerular cells	$GABA_AR$: α2, α5 subunits	Immunohistochemistry	Panzanelli et al., 2007
Dentate gyrus	Type B	$GABA_AR$: α5, β3, γ2	Pharmacology	Song et al., 2012a
	Dentate gyrus:	$GABA_AR$: α1, α2, α4, α5, δ	*In situ* hybridization	Heldt and Ressler, 2007; Hortnagl et al., 2013
	Granule cells	α2, δ	Immunogold labeling in EM	Wei et al., 2003

combinations α4β2δ and α5β3γ2 (Glykys et al., 2008). In the SGZ neural stem cells respond tonically to GABA via the α5β3γ2 $GABA_AR$ composition to control their quiescent condition (Song et al., 2012a). Further it has been shown that $GABA_ARs$ containing the α4 subunit are expressed in type I cells to control their proliferation rate (Duveau et al., 2011). In conclusion, a huge diversity of $GABA_ARs$ is present in the OB and DG, although it is still not clear how the signaling through the different $GABA_AR$ subtypes is processed. We will address the relevance of distinct $GABA_AR$ subtypes in modulating critical stages of adult neurogenesis.

Most of the studies described here are performed using RT-PCR or pharmacological approaches combined to KO mice. For most of the $GABA_AR$ subunits a clear evidence of pattern expression in precursors and neuroblasts is missing. To better understand the role of the different $GABA_AR$ subunits, a deeper investigation using immunohistochemistry using specific antibodies against the different $GABA_AR$ subunits and cell markers, would be useful.

GABA SIGNALING IN ADULT NEUROGENESIS

Adult neurogenesis in the OB and in the DG differs in many aspects. Neuroblasts proliferate in two different niches; they migrate along different routes and for different distances. Then, when neuroblasts incorporate in the preexisting circuitry, they integrate with a very different timing. The functional role of adult neurogenesis, although it is still not entirely clear, is different in the two systems. Nevertheless, GABAergic neurotransmission regulates the entire process of adult neurogenesis in both systems, suggesting common mechanisms, as well as possible differences, including certain functional specificities. In the following paragraph, we aim to compare the role of GABA in regulating adult neurogenesis in the OB and DG, focusing in particular on the contribution of major $GABA_AR$ subtypes to this process (See **Table 2**).

PROLIFERATION: GABA IS A STOP SIGNAL IN CELL PROLIFERATION

In the SVZ, precursors and neuroblasts are already sensitive to neurotransmitters (Berg et al., 2013). Neonatal SVZ progenitor cells show chloride currents activated by GABA and muscimol, but they are insensitive to ATP, kainate, NMDA, and ACh (Stewart et al., 2002). The absence of synapses at the EM level suggests that GABA is synthesized and released in a non-synaptic manner by neuroblasts. This conclusion is also supported by electrophysiological and IHC analyses (Doetsch et al., 1997). Moreover, electrophysiological studies have shown that tonic release of GABA activates $GABA_ARs$ expressed by neuroblasts and stem cells (Stewart et al., 2002; Wang et al., 2003; Bolteus and Bordey, 2004; Liu et al., 2005). A second source of GABAergic innervation comes from medium spiny or aspiny neurons from the striatum. The activation of striatal neurons increases calcium level into SVZ neuroblasts (Young et al., 2014). Moreover, acute treatment with muscimol decreases BrDU incorporation in type B and C cells of the SVZ (Fernando), and therefore decreases proliferation.

Still it is unknown whether $GABA_B$ receptors ($GABA_BRs$) are expressed in the SVZ. The high affinity GABA transporter GAT4 tightly regulates GABA levels in stem cells, but not in neuroblasts. Pharmacological inhibition of $GABA_AR$ in organotypic cultures *in vivo* induce increased proliferation. Conversely, inhibition of GAT4 produces the opposite effect (Liu et al., 2005).

In the SGZ, type I cells respond to tonic GABAergic stimulation (Tozuka et al., 2005; Ge et al., 2006). In the DG, γ2 subunit (presumably associated with the α5 subunit), has a unique role in maintaining adult precursor cells quiescence. Using a conditional transgenic γ2-KO mouse, Song et al. (2012a,b) found increases in proliferation and increases in symmetrical cell-renewal of type I cells. Interestingly, using an optogenetic approach they identify the source of GABAergic innervation in parvalbumin (PV) positive interneurons, but not somatostatin and vasoactive intestine polypeptide interneurons (Song et al., 2012a). Furthermore, α4 -KO mice showed an increase in proliferation as assessed using BrdU injection (Duveau et al., 2011). A mechanism by which GABA regulates proliferation is through regulation of epigenetic mechanisms that inhibits DNA synthesis (Fernando et al., 2011).

Together these results suggest that at least two distinct $GABA_AR$ subtypes control stem cells and neural precursor cells.

The role of $GABA_BR$ in type I cells was investigated by Felice et al. (2012). After blocking $GABA_BR$ with its antagonist

Table 2 | Comparison of the role of different GABA receptor and receptor subunits in different stages of mouse adult neurogenesis in the OB and DG.

		Gaba receptor	Model	Effect	References
PROLIFERATION	OB	$GABA_AR$	Pharmacological inhibition	Increase in proliferation	Liu et al., 2005
	DG	γ2 α4 $GABA_B1$	Conditional KO mice KO mice Pharmacological blocking and transgenic KO mouse	Decrease in proliferation of type I cells Increase in cell proliferation Increase in proliferation	Song et al., 2012b Duveau et al., 2011 Felice et al., 2012 Rakic, 2002
MIGRATION	OB	α4	KO mice	Delayed radial migration	Unpublished
	DG	α2 α4	KO mice KO mice	Increased distance of migration Impaired migration	Duveau et al., 2011 Duveau et al., 2011
SPECIFICATION	OB	NA	NA	NA	
	DG	$GABA_AR$ γ2	Agonist Conditional deletion	Increased number of new neurons Increased fraction of astrocytes	Tozuka et al., 2005 Song et al., 2012a
INTEGRATION	OB	α2	Transgenic mice and virus injection	Reduction of dendritic ramifications and spines	Pallotto et al., 2012
	DG	α2 α4	KO mice KO mice	Reduction of dendritic branching in mature cells Reduction of dendrite growth and branching	Duveau et al., 2011 Duveau et al., 2011
PLASTICITY	OB	α2	Transgenic mice and virus injection	Unable to restore spine density after enrichment	Pallotto et al., 2012
	DG	NA	NA	NA	

NA, not available.

CGP52432, they found an increase in cell proliferation in the ventral hippocampus after 21 days of treatment, but not after acute treatment with the drug. $GABA_BR$ is made up by different subunits $GABA_B1$ and $GABA_B2$ (Gassmann and Bettler, 2012). Giachino et al. (2014) demonstrated that $GABA_B1$-KO mice show an increased adult progenitor proliferation accompanied with an unaltered cell survival. Since $GABA_BR$ are expressed not only by GCs, these effects may be a result of an indirect effect.

In summary, $GABA_AR$ activation decreases the proliferation rate in both OB and DG. GABA concentrations modulate the number of neuroblasts generated by precursor cells, suggesting a negative feedback mechanism to maintain the balance between proliferation and migration (Bordey, 2007 rev).

MIGRATION: GABA DRIVES NEUROBLASTS MIGRATION

OB neuroblasts migrate a long distance, from the SVZ to the OB. They migrate tangentially to the SVZ and once within the OB, they migrate radially respect to the OB using a vascular scaffold (Bovetti et al., 2007). Many factors are involved in this process, and GABA seems to be an important modulator in the tangential migration process. Migrating neuroblasts in the RMS express a variety of $GABA_ARs$ in developmently-related combinations (Pathania et al., 2010). Ambient GABA in the RMS reduces the speed of neuroblast migration in acute slices of adult and juvenile mice. Blocking GABA transporters or enhancing GABA release from neuroblasts slows the speed of migration (Bolteus and Bordey, 2004). The migration speed is also regulated by the depolarizing effect of GABA. Mejia-Gervacio et al. (2011) silenced NKCC1 expression with a short harpin RNA strategy in OB acute slices, which did not allow them to make GABA depolarizing. They reported that NKCC1 activity is necessary for maintaining normal migratory speed and regulating the resting membrane potential of postnatal migratory neuroblasts. They also demonstrated that NKCC1 function is strongly reduced at the time in which the cells reach the GCL (Mejia-Gervacio et al., 2011). Conversely, in α4-KO mice, many neuroblasts that complete their tangential migration apparently fail to enter into the GCL, suggesting a role of tonic GABAergic transmission for regulating migration into the gray matter (Fritschy, unpublished).

In the DG, neuroblasts migrate only few microns from the neurogenic niche to their final location, and this process has consequently received less scrutiny. However, Duveau et al. (2011) showed that in α4-KO mice neuroblasts migrate a significantly shorter distance compared to wild type mice. Conversely an opposite phenotype was observed in α2-KO mice, where a higher number of neuroblasts migrate deeper into the GCL. In this example, GABA signaling has a dual and opposite role depending

on the receptor it acts on. This suggests that the intracellular pathway activated by the two different receptors may contribute to different functions. Moreover, activation of α4 subunit is critical modulating entry of neuroblasts into the GCL in both the DG and the OB.

DIFFERENTIATION

In the OB, it has been estimated that 50–75% of neuroblasts become GABAergic OB-GCs, while the remaining become PGCs. The fate of OB interneurons depends on genetic (Kohwi et al., 2005; Waclaw et al., 2006; Saino-Saito et al., 2007), spatial and temporal factors (for a review see Lledo et al., 2006). The role of $GABA_AR$-mediated transmission has yet to be investigated.

GABA is one of the major extrinsic factors regulating differentiation of Type II cells of the DG through $GABA_AR$. Indeed, administration of a $GABA_AR$ agonist significantly increased the number of new neurons labeled with BrdU, while the $GABA_A$ antagonist has no effect. Progenitors in the SGZ receive GABAergic, but not glutamatergic inputs. GABA induces the expression of NeuroD, a transcription factor that positively regulates neuronal differentiation toward DG-GCs (Tozuka et al., 2005).

Depolarizing action of GABA

One open question that has not been elucidated is the timing of when GABA switches from a depolarizing to a hyperpolarizing agent in adult-born neurons.

Using gramicidin perforated patch clump, Wang et al. (2003) demonstrated that in neuronal progenitors in the SVZ as well as migrating neuroblasts in the SVZ GABA has a depolarizing effect. Mejia-Gervacio et al. (2011) silenced NKCC1 expression with short a harpin RNA strategy in OB acute slices, to make GABA depolarizing. Although these data suggest a depolarizing role for GABA in perinatal neuroblast migration, it is still not clear when GABA switches from a depolarizing to a hyperpolarizing role during OB adult neurogenesis.

In the DG it is well established that GABA has a depolarizing effect on adult-born dentate GCs, recapitulating what happens during the ontogenic development. Ge et al. (2006) used a short harpin RNA strategy to silence NKCC1 activity. They found that the switch from GABA-induced depolarization occurs between 14 and 28 dpi (days post injection). More recently, Chancey et al. (2013) discovered that GABA depolarization is needed for AMPA receptor (AMPAR) synaptic incorporation in developing adult-born GCs.

INTEGRATION AND MATURATION

Many studies have described the development and integration of adult born neurons. Similarly, many reviews have been written to summarize these findings, for an in-depth analysis (see Petreanu and Alvarez-Buylla, 2002; van Praag et al., 2002; Carleton et al., 2003; Lledo et al., 2006; Kelsch et al., 2008, 2010; Dieni et al., 2012; Gu et al., 2013; Platel and Kelsch, 2013). Here, we want to review the role of $GABA_AR$ and $GABA_AR$ subunits in modulating integration and maturation of adult born neurons.

Structural development of adult-born neurons in the OB

eGFP lentiviral injection into the RMS to birth-date adult-born OB-GCs, were used to described GCs development (Petreanu and Alvarez-Buylla, 2002; Carleton et al., 2003; Pallotto et al., 2012). When neuroblasts arrive into the OB after having migrated along the RMS, they exhibit the typical bipolar morphology of migrating cells. According to Alvarez-Buylla classification, we can distinguish 5 different classes of adult born neurons according to their morphology. Class 1 cells are neuroblasts migrating tangentially toward the OB (observed 2–7 days after virus injection—dpi). Class 2 neurons leave the RMS and migrate radially in the OB (5–7 dpi). Class 3 neurons extend a simple apical dendrite toward the mitral cell layer (MCL) (9–13 dpi). In class 4 neurons, the apical dendrite has crossed the MCL and starts branching within the EPL (11–22 dpi). Finally, class neurons 5 are considered morphologically mature GCs with spiny apical dendrites branched in the EPL (from 15 dpi) (Alvarez-Buylla et al., 2001).

Similarly, adult-born GCs have a unique sequence of electrophysiological maturation. Migratory cells (stages 1 and 2) have membrane properties similar to immature precursors, and do not show spontaneous postsynaptic currents. In contrast, stages 3–5 neurons start to show excitatory and inhibitory postsynaptic currents (Carleton et al., 2003).

Pallotto et al. further described the maturation of adult-born GCs. Dendritic growth and ramification was monitored from 7 to 90 dpi (Pallotto et al., 2012). Sholl analysis on developing adult-born OB-GCs demonstrates that the cells reach a maximum in dendritic branching after 30 dpi, thin value decreasing at 90 dpi. A similar pruning has been shown also for dendritic spines. It has been reported that a maximum dendritic spine density in the EPL at 28 dpi when injecting in the SVZ and at 30 dpi when injecting in the RMS (Whitman and Greer, 2007; Pallotto et al., 2012). Similarly, adult-born PGCs undergo dendritic spine pruning, reaching the maximum spine density between 1 and 3 months post-injection (Livneh and Mizrahi, 2011). These authors suggest that dendritic structure is determined by animal age rather than neuronal age. Altogether these findings demonstrate that the integration of adult-born OB interneurons is a long process and that external factors play a role in shaping the adult morphology of the cell through a pruning mechanism.

Using the Cre-lox system to selectively silence α2-$GABA_AR$ in virally-transfected cells, Pallotto et al. (2012) investigated the role of phasic GABAergic transmission into adult-born OB-GCs. Inactivation of the α2 subunit gene has detrimental effects on adult-born GCs structural maturation. The authors showed that α2-KO cells have reduced dendritic branching and a number of reduced spines when compared to the WTs. Therefore, the presence of postsynaptic α2-$GABA_ARs$ is fundamental for the growth of dendrites and spines observed in WT mice.

Synaptic development in adult-born neurons in the OB

Adult-born OB-GCs are rapidly targeted by axon terminals as soon as they reach their final position in the GCL (Whitman and Greer, 2007; Kelsch et al., 2008; Panzanelli et al., 2009). GABAergic and glutamatergic contacts form on the dendrites and cell bodies within 3 dpi in the RMS and on apical dendrites only 1 day later, as shown by IHC quantification of synaptic puncta for

inhibitory and excitatory synapse markers. In addition, these contacts are already functional, as shown by whole cell patch clamp recordings (Panzanelli et al., 2009).

Quantification of synaptic inputs onto newborn OB-GCs at early stages of development (3–7 dpi) indicates initially a higher fraction of gephyrin positive puncta then PDS95. At 7 dpi, PDS95 clusters were predominant, suggesting a slight delay in the formation of glutamatergic contacts on newborn GCs. Absence of phasic GABAergic inputs though removal of the α2 subunit led to a marked reduction in spontaneous and evoked inhibitory post-synaptic currents (IPSCs). At the molecular level, the loss of α2 subunit is followed by a strong reduction of its scaffolding protein gephyrin. Presynaptic terminals were not affected and no compensatory effects by α3-$GABA_A$Rs, also expressed by OB-GCs, were evident. The reduced synaptic GABAergic function inputs also affected the development of glutamatergic contacts. After deletion of α2 subunit in adult-born GCs, Pallotto et al. (2012) found a reduction of glutamatergic synapses demonstrated by a decrease in spontaneous excitatory postsynaptic currents (EPSCs), and a reduction in the PDS95 positive puncta on the spine head.

Development of adult-born neurons in the DG

Within 4 weeks after symmetric division, newborn DG-GCs extend their dendrites into the molecular layer, they first receive slow GABAergic inputs from hilar interneurons and from Ivy cells (Deshpande et al., 2013). Later, they receive numerous glutamatergic inputs from lateral and medial perforant pathways, and lastly perisomatic GABA synapses from various types of basket cells [parvalbumin or cholecystokinin- expressing cells (Katona et al., 1999; Song et al., 2013)], as well as axo-axonic contacts from chandelier cells. Adult-born GC axon projections reach the *stratum lucidum* of the CA3 region as well as the *stratum oriens*, where they form mossy fiber terminals (Esposito et al., 2005; Overstreet Wadiche et al., 2005; Toni et al., 2007; Jessberger et al., 2008; Markwardt and Overstreet-Wadiche, 2008; Zhao et al., 2010). From recent literature it is emerging that another important role of GABA neurotransmission is in the development of DG-GCs. Since GCs express different $GABA_A$R subunits, Duveau et al. (2011) dissected the role of α2– and α4-containing $GABA_A$R using lentivirus injection in KO mice. At 14 dpi α4-KO mice show a significant reduction of dendritic ramification, whereas the initial growth of dendrites was normal in α2- and δ-KO. At later stages (42 dpi) also α2-KO has a reduced dendritic complexity suggesting that the two different $GABA_A$R subunits have different roles in the dendritic tree development. However, it is important to note that DG-GCs also receive phasic inhibition by α1-$GABA_A$R (Sun et al., 2004), therefore, the deletion of the α2 subunit may be compensated for, and may not cause a complete loss of GABAergic synaptic inputs onto adult-born GCs.

PLASTICITY AND CRITICAL PERIOD

Adult-born GC development described above is not only regulated by an intrinsic program or local signaling molecules. The behavior of the neurons is also affected by their cellular age and by changes in the local environment. The term "critical period" is widely used to describe a specific time window in which neuronal proprieties are particularly prone to modification by external stimuli or experience. Consequences of the critical period are an enhanced morphological and synaptic plasticity that may shape behaviors.

Nissant et al. (2009) demonstrated the tendency of adult-born OB-GCs to undergo long-term potentiation (LTP) after their arrival in the bulb. The ability to undergo LTP faded as newborn neurons matured. LTP is the leading candidate mechanism for memory encoding and the presence of LTP only in a defined "time window" (around 20–30 dpi) suggests that newborn GCs are particularly sensitive to synaptic plasticity (Nissant et al., 2009). External stimuli shape the final morphology of OB-GCs acting on synaptic connectivity. Pallotto et al. (2012) documented that adult-born GCs that were subjected to odor enrichment showed increased spine density. This indicates that by controlling odor exposure during a "critical period," it is possible to control the level of excitatory drive onto GCs trough principal cell activation. The increase in spine density is most likely due to a stabilization of synaptic turnover rate (Livneh and Mizrahi, 2012).

Varying the degree of sensory inputs to the OB, using olfactory enriched environment or depriving sensory stimuli, Pallotto et al. (2012) found that none of the two treatments caused significant changes in spine density in adult-born OB-GCs lacking the α2-$GABA_A$R subunit. This observation indicates that GABAergic synaptic transmission mediated by α2-$GABA_A$Rs is required for structural adaptations of adult-born GCs in response to sensory challenges during the phase of dendritic/spine exuberance. GABA neurotransmission may be a candidate to regulate the opening/closing of the critical period in adult neurogenesis in the OB.

Between 28 and 42 days after birth, adult-born DG-GCs show a critical period with enhanced LTP (Ge et al., 2007a; Marin-Burgin et al., 2012). In this time window, they have different mechanisms that make them hypersensitive to stimuli. For example, adult-born GCs have lower activation threshold due to an enhanced E/I balance (Marin-Burgin et al., 2012). Furthermore, adult-born DG-GCs express NR2B NMDA receptor (NMDAR) subunit that appears to be associated with enhanced plasticity (Ge et al., 2007a).

Interestingly, the critical period is preceded by the transition of GABA from excitatory to inhibitory (Ge et al., 2006). Recently, Chancey et al. (2013) thoroughly investigated the role of GABAergic depolarization during the critical period. They found that GABAergic synaptic depolarization enables activation of NMDAR in the absence of AMPAR-mediated transmission *in vitro* as well as *in vivo* after a brief exposure to enriched environment. Therefore, GABAergic depolarization is required and allows excitatory synapse un-silencing that is induced by activity.

SURVIVAL

Both in OB and DG, a massive number of adult-born neurons undergo programmed cell death within a month after birth [specifically, 50% in the OB (Petreanu and Alvarez-Buylla, 2002) and 60–80% in the DG (Cameron and McKay, 2001)]. While deletion of some $GABA_A$R subunits affect morphology and plasticity of adult-born cells (see previous paragraphs), it does not explicitly affect survival. This raises the question: is morphological

maturation unrelated to survival? According to Pallotto, Duveau and Giachino deletion of different $GABA_AR$ and $GABA_BR$ subunits do not affect neuroblasts survival (Rakic, 2002; Duveau et al., 2011; Pallotto et al., 2012). However, up to now, few studies explored properly the role of GABA signaling in the survival of adult-born cells. It would be interesting to use specific markers, like caspase or ssDNA antibodies, to detect the cell death rate in adult-born neurons in OB and DG in mice lacking specific subunits of $GABA_AR$.

BEYOND GABAergic SIGNALING

The formation of a functional network in the CNS requires cell integration, synapse formation and maturation through the orchestration of several factors. Understanding these processes is a major challenge in the neuroscience field. Adult neurogenesis is a formidable tool for this purpose. Adult-born cells are rapidly targeted by axon terminals, forming functional excitatory and inhibitory contacts. Considering that during the first month of development, about half of the adult-born cells die (Cameron and McKay, 2001; Winner et al., 2002), synapse formation could represent a way by which GCs are positively selected. Soon after the initial integration step, adult-born GCs go through a period of prominent structural reorganization involving changes in dendritic arborization and spine density (Whitman and Greer, 2007; Livneh and Mizrahi, 2011; Pallotto et al., 2012) (**Figure 2**).

In both OB and DG, adult-born neurons show a time window in which adult-born neurons are more susceptible to stimuli, showing an enhanced LTP (**Figure 2**). In the OB, absence of phasic GABAergic input, mediated by α2-$GABA_ARs$, makes adult-born GCs incapable of responding to external stimuli. In the DG, GABAergic activity allows synapse unsilencing driving AMPAR insertion (Chancey et al., 2013). However, the molecular mechanism by which GABA exerts its function is still not clear.

If there is a huge variety of $GABA_AR$ each of them modulating a different function in adult neurogenesis, then there must be heterogeneous downstream pathways. Here we review three pathways that have been shown to be involved in the downstream molecular signaling. Those pathways may be independent or they may converge and interact at any level.

SECOND MESSENGERS: EXAMPLES FROM CREB AND PI3K SIGNALING PATHWAY

Another common mechanism in the OB and DG by which GABAergic transmission affect maturation and the critical period of adult-born neurons could be the cAMP response element-binding protein (CREB) pathway (Merz et al., 2011). CREB is a transcription factor involved in many different aspects of adult neurogenesis (Lonze and Ginty, 2002). CREB and its active form, pCREB is expressed in the SVZ and by adult-born neurons in the OB. pCREB is only sporadically expressed by DCX^+ neuroblasts in the SVZ, but is strongly present in migrating neuroblasts in the RMS and OB (Herold et al., 2011). The loss of CREB function results in a reduction of the survival rate of newborn neurons and impairs morphological differentiation (Giachino et al., 2005; Herold et al., 2011). Similarly, in the DG the timing of pCREB expression is highly regulated and present in DCX+ cells. In a cell autonomous manner, CREB signaling pathway regulates survival and morphological development (Jagasia et al., 2009).

pCREB controls multiple steps including proliferation, survival, neurite outgrowth and dendrite branching. Formation of spines accompanied with glutamatergic inputs occurs at later stage (28 days after birth), suggesting that GABA might trigger the signaling cascade leading to the phosphorylation of CREB (Magill et al., 2010).

An interesting link between GABAergic activity and CREB signaling come from the work of Jagasia et al. In this work, using an *in vivo* approach with retroviral injection in the DG, the authors demonstrate that CREB phosphorylation and signaling affect survival and maturation and integration of adult born DG-GCs. The peak of CREB phosphorylation occurs when GABA acts with a depolarizing effect on adult born GCs. Using a shNKCC1 virus to ablate the depolarizing effect of GABA, the authors showed that impairment in morphological maturation and differentiation and a reduced survival rate can be rescued by CREB activation (Jagasia et al., 2009).

shRNA against γ2 subunit in adult-born DG-GCs causes a reduced dendritic growth. A similar phenotype is also seen in mice where NKCC1 is down regulated by shRNA (Kim et al., 2012). The selective deletion of the α2 and α4 - subunit-containing $GABA_ARs$ in adult-born -GCs, alters dendritogenesis but not survival suggesting that there might be another $GABA_AR$ involved in CREB phosphorylation during the differentiation of adult-born neurons. This different $GABA_AR$ subunit may regulate the survival but not the development or integration of adult born GCs (Duveau et al., 2011). Despite the lack of strong evidences of $GABA_AR$ involvement in survival, this phenomenon is not deeply studied. Therefore, we cannot exclude a role of other $GABA_AR$ subunits having a role in survival of adult-born neurons.

Epigenetic modifications elicited by $GABA_AR$ activation also influence cell proliferation in the SVZ (Fernando et al., 2011). An interesting paper from Fernando et al. showed *in vitro* and *in vivo* that acute pharmacological modulation of $GABA_AR$ with muscimol or bicuculline leads to increase or decrease of histone H2AX, respectively. As a consequence, the authors observed a decrease or an increase of BrDU incorporation from type C and B cells of the SVZ. Pharmacological modulation of $GABA_AR$, therefore affects proliferation. This effect is also observed long-term after chronic pharmacological treatment, and it affects the number of BrDU positive GCs in the GCL. In a previous paper from the same group, it was demonstrated in embryonic stem cells that $GABA_AR$ signals through phosphatidylinositol-3-OH kineases to phosphorylate the histone variant H2AX (Andang et al., 2008). Together, these works indicate that $GABA_AR$ modulate proliferation thought an epigenetic mechanism, that may have important consequence for long-term modulation of the neural niche size and composition, and therefore of the adult neuronal cells that are produced.

EXTRASYNAPTIC RECEPTORS MEDIATE THEIR EFFECTS VIA CA^{2+} SIGNALING

E/I balance has a fundamental role in neuron development. Alterations of the E/I balance cause defects such as impairment

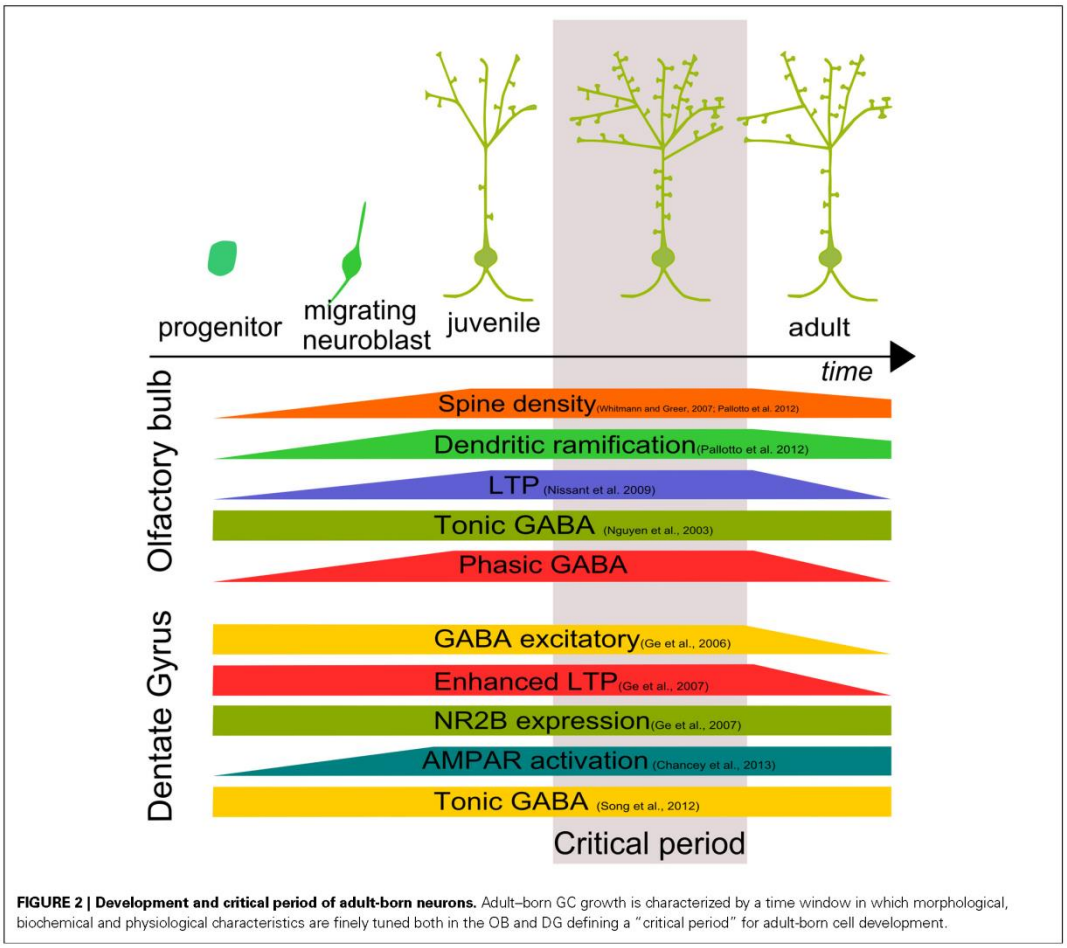

FIGURE 2 | Development and critical period of adult-born neurons. Adult–born GC growth is characterized by a time window in which morphological, biochemical and physiological characteristics are finely tuned both in the OB and DG defining a "critical period" for adult-born cell development.

of dendritic growth during both in the juvenile and mature development (Cancedda et al., 2007; Ge et al., 2007b). During development, when GABA has an excitatory role, GABAergic-mediated Ca^{2+} signaling mediates several aspects such as migration and maturation. GABA neurotransmitter increase $[Ca_i^{2+}]$ influx thought voltage-sensitive channels (Yuste and Katz, 1991). In cortical development, chemotaxis and chemokinesis are mediated by an increase in intracellular Ca^{2+} concentration stimulated by GABA (Behar et al., 1996). In type A progenitor SVZ cells, $GABA_AR$ activation induce variation in $[Ca_i^{2+}]$ to modulate proliferation (Nguyen et al., 2003). Similarly, in SVZ-derived neurons, an exposure of 10–30 s to GABA induces $[Ca_i^{2+}]$ increase. $GABA_AR$ activation is dependent of L-type voltage-gated Ca^{2+} channels (Gascon et al., 2006). In developing DG-GCs, GABA induces Ca^{2+} transients via L-type Ca^{2+} channels. These Ca^{2+} transients are important for suppressing axonal but not dendritic growth (Lee et al., 2012). In the adult brain, spine shrinkage and elimination are promoted by activation of $GABA_AR$ occurring after an action potential. In this particular case, GABAergic inhibition suppresses local dendritic Ca^{2+} transient that promotes competitive selection of dendritic spines (Hayama et al., 2013). In the DG, GABAergic hippocampal activity depolarizes type-2 cells leading to an increase in intracellular Ca^{2+} concentration and promoting activity-dependent neuronal differentiation. In the SVZ, $GABA_AR$ induces depolarization leading to the opening of L-type Ca^{2+} channels (Young et al., 2010). In SVZ precursor cells, the presence of tonic currents has been reported (Liu et al., 2005; Bordey, 2007). These data support the notion that Ca^{2+} dynamics are regulated by tonic $GABA_AR$ activity at early stage of neuronal development; however phasic GABAergic activation cannot be excluded.

SYNAPTIC RECEPTORS AND GEPHYRIN SIGNAL

GABAergic signals are mediated by both synaptic and extrasynaptic receptors. The presence of synaptic and extrasynaptic $GABA_A$Rs on the plasma membrane is a highly dynamic state is and regulated by multiple mechanisms influencing the position and properties of the receptor, including interactions with the gephyrin scaffold. Gephyrin mostly regulates the clustering of synaptic $GABA_A$Rs. Gephyrin is crucial for the formation of GABAergic synapses, but it also interacts with other signaling molecules. $GABA_A$R-gephyrin interactions regulate gephyrin's clustering properties and/or are anchored at GABAergic synapses by binding to gephyrin. *In vitro* work emphasizes the importance of gephyrin phosphorylation in regulainge GABAergic synaptic function (Tyagarajan and Fritschy, 2010; Tyagarajan et al., 2011, 2013). In particular, inhibiting phosphorylation of the residue Ser270 of gephyrin leads to an upregulation of postsynaptic gephyrin clusters, and consequently to an increase in the frequency and amplitude of mini GABAergic currents (Tyagarajan et al., 2011). Conversely, a mutation in a surface-exposed loop (L2B) prevents gephyrin from clustering and prevents the formation of GABAergic postsynaptic densities (Lardi-Studler et al., 2007). This suggests that the ability of gephyrin in modulating GABAergic synaptogenesis can have a direct influence on the stabilization of $GABA_A$Rs in a phosphorylation-dependent manner or on downstream signaling cascades. Downstream gephyrin signaling involves adhesion molecules such as neuroligin 2, or GDP/GTP exchange factors (GEFs) such as collybistin, and also small Rho- GTPases as Cdc-42 or profiling (for a review see Vadodaria and Jessberger, 2013). Cdc-42 can modulate actin and microtubules. This signaling may play a role in maturation and plasticity of adult-born GCs by interacting with the cytoskeleton. Therefore, the morphological deficits seen in adult-born OB-GCs in α2-KO might be due to the fact that gephyrin clusters are disrupted (Pallotto et al., 2012). This presumably leads to a dispersion of signaling molecules such as collybistin and Cdc-42 away from the synapses. In particular, this possibility implies that the reduction of spine density and dendritic growth in adult-born OB-GCs, which has been observed in Pallotto et al. (2012) after selective depletion of α2 subunit, might be due to impaired gephyrin clustering (Pallotto et al., 2012).

To better understand the role of the downstream signal pathway of gephyrin, and therefore its role as scaffold for a proper adult-born cell maturation and integration, it would be interesting to study its different states of phosphorylation and expression patterns.

In summary, GABAergic activity exerts a variety of functions in adult neurogenesis. The role of the neurotransmitter is defined by a variety of $GABA_A$R subunits. Sometimes, two different $GABA_A$R subunits exert different and opposite effects. For example in the DG, the α2 and α4 subunits have an opposing effect on cell migration (Duveau et al., 2011) (for a comparison see **Table 1**). Multiple pathways may mediate the specific effects of the different subunits. Here, we have only described a subset of these molecular pathways, which are intermingled, making it difficult to determine their specific roles. For example, it is possible that GABA induces influx of Ca^{2+} in newborn GCs leading to CREB induced gene expression. Similarly, Ca^{2+} may promote gephyrin phosphorylation. It would be interesting to understand how GABAergic synaptic and extrasynaptic receptor activity is orchestrated. Although it is known that different $GABA_A$R subtypes modulate different aspects of adult neurogenesis, which downstream pathways are involved is still unclear.

Adult neurogenesis provides neuroblasts in a mature network. It is a unique feature that neuroblasts have to integrate into already existing network, lacking all the neurotrophic factors that are present during brain development. We still need to understand which mechanisms and which receptors GABA used to modulate the intracellular pathways that leads neuroblasts to integrate and mature. Beyond the fascinating quest to understand the mechanisms that drive adult neurogenesis, unraveling this issue will help us better understand not only brain function and development but also neurodevelopmental disorders. This may contribute to new strategies for cell replacement therapies.

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REFERENCES

Abrous, D. N., Koehl, M., and Le Moal, M. (2005). Adult neurogenesis: from precursors to network and physiology. *Physiol. Rev.* 85, 523–569. doi: 10.1152/physrev.00055.2003

Alvarez-Buylla, A., Garcia-Verdugo, J. M., and Tramontin, A. D. (2001). A unified hypothesis on the lineage of neural stem cells. *Nat. Rev. Neurosci.* 2, 287–293. doi: 10.1038/35067582

Andang, M., Hjerling-Leffler, J., Moliner, A., Lundgren, T. K., Castelo-Branco, G., Nanou, E., et al. (2008). Histone H2AX-dependent GABA(A) receptor regulation of stem cell proliferation. *Nature* 451, 460–464. doi: 10.1038/nature06488

Behar, T. N., Li, Y. X., Tran, H. T., Ma, W., Dunlap, V., Scott, C., et al. (1996). GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms. *J. Neurosci.* 16, 1808–1818.

Berg, D. A., Belnoue, L., Song, H., and Simon, A. (2013). Neurotransmitter-mediated control of neurogenesis in the adult vertebrate brain. *Development* 140, 2548–2561. doi: 10.1242/dev.088005

Bolteus, A. J., and Bordey, A. (2004). GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. *J. Neurosci.* 24, 7623–7631. doi: 10.1523/JNEUROSCI.1999-04.2004

Bordey, A. (2007). Enigmatic GABAergic networks in adult neurogenic zones. *Brain Res. Rev.* 53, 124–134. doi: 10.1016/j.brainresrev.2006.07.004

Bovetti, S., Gribaudo, S., Puche, A. C., De Marchis, S., and Fasolo, A. (2011). From progenitors to integrated neurons: role of neurotransmitters in adult olfactory neurogenesis. *J. Chem. Neuroanat.* 42, 304–316. doi: 10.1016/j.jchemneu.2011.05.006

Bovetti, S., Hsieh, Y. C., Bovolin, P., Perroteau, I., Kazunori, T., and Puche, A. C. (2007). Blood vessels form a scaffold for neuroblast migration in the adult olfactory bulb. *J. Neurosci.* 27, 5976–5980. doi: 10.1523/JNEUROSCI.0678-07.2007

Cameron, H. A., and McKay, R. D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* 435, 406–417. doi: 10.1002/cne.1040

Cancedda, L., Fiumelli, H., Chen, K., and Poo, M. M. (2007). Excitatory GABA action is essential for morphological maturation of cortical neurons *in vivo*. *J. Neurosci.* 27, 5224–5235. doi: 10.1523/JNEUROSCI.5169-06.2007

Carleton, A., Petreanu, L. T., Lansford, R., Alvarez-Buylla, A., and Lledo, P. M. (2003). Becoming a new neuron in the adult olfactory bulb. *Nat. Neurosci.* 6, 507–518. doi: 10.1038/nn1048

Chancey, J. H., Adlaf, E. W., Sapp, M. C., Pugh, P. C., Wadiche, J. I., and Overstreet-Wadiche, L. S. (2013). GABA depolarization is required for

experience-dependent synapse unsilencing in adult-born neurons. *J. Neurosci.* 33, 6614–6622. doi: 10.1523/JNEUROSCI.0781-13.2013

Deshpande, A., Bergami, M., Ghanem, A., Conzelmann, K. K., Lepier, A., Gotz, M., et al. (2013). Retrograde monosynaptic tracing reveals the temporal evolution of inputs onto new neurons in the adult dentate gyrus and olfactory bulb. *Proc. Natl. Acad. Sci. U.S.A.* 110, E1152–E1161. doi: 10.1073/pnas.1218991110

Dieni, C. V., Chancey, J. H., and Overstreet-Wadiche, L. S. (2012). Dynamic functions of GABA signaling during granule cell maturation. *Front. Neural Circuits* 6:113. doi: 10.3389/fncir.2012.00113

Doetsch, F., Garcia-Verdugo, J. M., and Alvarez-Buylla, A. (1997). Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* 17, 5046–5061.

Duveau, V., Laustela, S., Barth, L., Gianolini, F., Vogt, K. E., Keist, R., et al. (2011). Spatiotemporal specificity of GABAA receptor-mediated regulation of adult hippocampal neurogenesis. *Eur. J. Neurosci.* 34, 362–373. doi: 10.1111/j.1460-9568.2011.07782.x

Esposito, M. S., Piatti, V. C., Laplagne, D. A., Morgenstern, N. A., Ferrari, C. C., Pitossi, F. J., et al. (2005). Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.* 25, 10074–10086. doi: 10.1523/JNEUROSCI.3114-05.2005

Essrich, C., Lorez, M., Benson, J. A., Fritschy, J. M., and Luscher, B. (1998). Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. *Nat. Neurosci.* 1, 563–571. doi: 10.1038/2798

Felice, D., O'Leary, O. F., Pizzo, R. C., and Cryan, J. F. (2012). Blockade of the GABA(B) receptor increases neurogenesis in the ventral but not dorsal adult hippocampus: relevance to antidepressant action. *Neuropharmacology* 63, 1380–1388. doi: 10.1016/j.neuropharm.2012.06.066

Fernando, R. N., Eleuteri, B., Abdelhady, S., Nussenzweig, A., Andang, M., and Ernfors, P. (2011). Cell cycle restriction by histone H2AX limits proliferation of adult neural stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5837–5842. doi: 10.1073/pnas.1014993108

Fritschy, J. M., Benke, D., Johnson, D. K., Mohler, H., and Rudolph, U. (1997). GABAA-receptor alpha-subunit is an essential prerequisite for receptor formation *in vivo*. *Neuroscience* 81, 1043–1053. doi: 10.1016/S0306-4522(97)00244-3

Fritschy, J. M., Panzanelli, P., and Tyagarajan, S. K. (2012). Molecular and functional heterogeneity of GABAergic synapses. *Cell. Mol. Life Sci.* 69, 2485–2499. doi: 10.1007/s00018-012-0926-4

Gage, F. H. (2000). Mammalian neural stem cells. *Science* 287, 1433–1438. doi: 10.1126/science.287.5457.1433

Gascon, E., Dayer, A. G., Sauvain, M. O., Potter, G., Jenny, B., De Roo, M., et al. (2006). GABA regulates dendritic growth by stabilizing lamellipodia in newly generated interneurons of the olfactory bulb. *J. Neurosci.* 26, 12956–12966. doi: 10.1523/JNEUROSCI.4508-06.2006

Gassmann, M., and Bettler, B. (2012). Regulation of neuronal GABA(B) receptor functions by subunit composition. *Nat. Rev. Neurosci.* 13, 380–394. doi: 10.1038/nrn3249

Ge, S., Goh, E. L., Sailor, K. A., Kitabatake, Y., Ming, G. L., and Song, H. (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 439, 589–593. doi: 10.1038/nature04404

Ge, S., Pradhan, D. A., Ming, G. L., and Song, H. (2007b). GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci.* 30, 1–8. doi: 10.1016/j.tins.2006.11.001

Ge, S., Yang, C. H., Hsu, K. S., Ming, G. L., and Song, H. (2007a). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* 54, 559–566. doi: 10.1016/j.neuron.2007.05.002

Giachino, C., Barz, M., Tchorz, J. S., Tome, M., Gassmann, M., Bischofberger, J., et al. (2014). GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. *Development* 141, 83–90. doi: 10.1242/dev.102608

Giachino, C., De Marchis, S., Giampietro, C., Parlato, R., Perroteau, I., Schutz, G., et al. (2005). cAMP response element-binding protein regulates differentiation and survival of newborn neurons in the olfactory bulb. *J. Neurosci.* 25, 10105–10118. doi: 10.1523/JNEUROSCI.3512-05.2005

Glykys, J., Mann, E. O., and Mody, I. (2008). Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? *J. Neurosci.* 28, 1421–1426. doi: 10.1523/JNEUROSCI.4751-07.2008

Gu, Y., Janoschka, S., and Ge, S. (2013). Neurogenesis and hippocampal plasticity in adult brain. *Curr. Top. Behav. Neurosci.* 15, 31–48. doi: 10.1007/7854_2012_217

Hayama, T., Noguchi, J., Watanabe, S., Takahashi, N., Hayashi-Takagi, A., Ellis-Davies, G. C., et al. (2013). GABA promotes the competitive selection of dendritic spines by controlling local Ca2+ signaling. *Nat. Neurosci.* 16, 1409–1416. doi: 10.1038/nn.3496

Heldt, S. A., and Ressler, K. J. (2007). Forebrain and midbrain distribution of major benzodiazepine-sensitive GABAA receptor subunits in the adult C57 mouse as assessed with *in situ* hybridization. *Neuroscience* 150, 370–385. doi: 10.1016/j.neuroscience.2007.09.008

Herold, S., Jagasia, R., Merz, K., Wassmer, K., and Lie, D. C. (2011). CREB signalling regulates early survival, neuronal gene expression and morphological development in adult subventricular zone neurogenesis. *Mol. Cell. Neurosci.* 46, 79–88. doi: 10.1016/j.mcn.2010.08.008

Hortnagl, H., Tasan, R. O., Wieselthaler, A., Kirchmair, E., Sieghart, W., and Sperk, G. (2013). Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain. *Neuroscience* 236, 345–372. doi: 10.1016/j.neuroscience.2013.01.008

Jacob, T. C., Moss, S. J., and Jurd, R. (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* 9, 331–343. doi: 10.1038/nrn2370

Jagasia, R., Steib, K., Englberger, E., Herold, S., Faus-Kessler, T., Saxe, M., et al. (2009). GABA-cAMP response element-binding protein signaling regulates maturation and survival of newly generated neurons in the adult hippocampus. *J. Neurosci.* 29, 7966–7977. doi: 10.1523/JNEUROSCI.1054-09.2009

Jessberger, S., Toni, N., Clemenson, G. D. Jr., Ray, J., and Gage, F. H. (2008). Directed differentiation of hippocampal stem/progenitor cells in the adult brain. *Nat. Neurosci.* 11, 888–893. doi: 10.1038/nn.2148

Kaila, K., Rydqvist, B., Pasternack, M., and Voipio, J. (1992). Inward current caused by sodium-dependent uptake of GABA in the crayfish stretch receptor neurone. *J. physiol.* 453, 627–645.

Katona, I., Acsady, L., and Freund, T. F. (1999). Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus. *Neuroscience* 88, 37–55. doi: 10.1016/S0306-4522(98)00302-9

Kelsch, W., Lin, C. W., and Lois, C. (2008). Sequential development of synapses in dendritic domains during adult neurogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16803-16808. doi: 10.1073/pnas.0807970105

Kelsch, W., Sim, S., and Lois, C. (2010). Watching synaptogenesis in the adult brain. *Ann. Rev. Neurosci.* 33, 131–149. doi: 10.1146/annurev-neuro-060909-153252

Kilb, W., Kirischuk, S., and Luhmann, H. J. (2013). Role of tonic GABAergic currents during pre- and early postnatal rodent development. *Front. Neural Circuits* 7:139. doi: 10.3389/fncir.2013.00139

Kim, J. Y., Liu, C. Y., Zhang, F., Duan, X., Wen, Z., Song, J., et al. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148, 1051–1064. doi: 10.1016/j.cell.2011.12.037

Kneussel, M., Brandstatter, J. H., Laube, B., Stahl, S., Muller, U., and Betz, H. (1999). Loss of postsynaptic GABA(A) receptor clustering in gephyrin-deficient mice. *J. Neurosci.* 19, 9289–9297.

Kohwi, M., Osumi, N., Rubenstein, J. L., and Alvarez-Buylla, A. (2005). Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. *J. Neurosci.* 25, 6997–7003. doi: 10.1523/JNEUROSCI.1435-05.2005

Kralic, J. E., Sidler, C., Parpan, F., Homanics, G. E., Morrow, A. L., and Fritschy, J. M. (2006). Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice. *J. Comp. Neurol.* 495, 408–421. doi: 10.1002/cne.20866

Lardi-Studler, B., Smolinsky, B., Petitjean, C. M., Koenig, F., Sidler, C., Meier, J. C., et al. (2007). Vertebrate-specific sequences in the gephyrin E-domain regulate cytosolic aggregation and postsynaptic clustering. *J. Cell Sci.* 120(Pt 8), 1371–1382. doi: 10.1242/jcs.003905

Lee, H., Lee, D., Park, C. H., Ho, W. K., and Lee, S. H. (2012). GABA mediates the network activity-dependent facilitation of axonal outgrowth from the newborn granule cells in the early postnatal rat hippocampus. *Eur. J. Neurosci.* 36, 2743–2752. doi: 10.1111/j.1460-9568.2012.08192.x

Levi, S., Logan, S. M., Tovar, K. R., and Craig, A. M. (2004). Gephyrin is critical for glycine receptor clustering but not for the formation of functional GABAergic synapses in hippocampal neurons. *J. Neurosci.* 24, 207–217. doi: 10.1523/JNEUROSCI.1661-03.2004

Liu, X., Wang, Q., Haydar, T. F., and Bordey, A. (2005). Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat. Neurosci.* 8, 1179–1187. doi: 10.1038/nn1522

Livneh, Y., and Mizrahi, A. (2011). Long-term changes in the morphology and synaptic distributions of adult-born neurons. *J. Comp. Neurol.* 519, 2212–2224. doi: 10.1002/cne.22625

Livneh, Y., and Mizrahi, A. (2012). Experience-dependent plasticity of mature adult-born neurons. *Nat. Neurosci.* 15, 26–28. doi: 10.1038/nn.2980

Lledo, P. M., Alonso, M., and Grubb, M. S. (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat. Rev. Neurosci.* 7, 179–193. doi: 10.1038/nrn1867

Lois, C., and Alvarez-Buylla, A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc. Natl. Acad. Sci. U.S.A.* 90, 2074–2077. doi: 10.1073/pnas.90.5.2074

Lois, C., and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* 264, 1145–1148. doi: 10.1126/science.8178174

Lois, C., Garcia-Verdugo, J. M., and Alvarez-Buylla, A. (1996). Chain migration of neuronal precursors. *Science* 271, 978–981. doi: 10.1126/science.271.5251.978

Lonze, B. E., and Ginty, D. D. (2002). Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35, 605–623. doi: 10.1016/S0896-6273(02)00828-0

Luskin, M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* 11, 173–189. doi: 10.1016/0896-6273(93)90281-U

Macdonald, R. L., and Olsen, R. W. (1994). GABAA receptor channels. *Ann. Rev. Neurosci.* 17, 569–602. doi: 10.1146/annurev.ne.17.030194.003033

Magill, S. T., Cambronne, X. A., Luikart, B. W., Lioy, D. T., Leighton, B. H., Westbrook, G. L., et al. (2010). microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20382–20387. doi: 10.1073/pnas.1015691107

Marin-Burgin, A., Mongiat, L. A., Pardi, M. B., and Schinder, A. F. (2012). Unique processing during a period of high excitation/inhibition balance in adult-born neurons. *Science* 335, 1238–1242. doi: 10.1126/science.1214956

Markwardt, S., and Overstreet-Wadiche, L. (2008). GABAergic signalling to adult-generated neurons. *J. Physiol.* 586, 3745–3749. doi: 10.1113/jphysiol.2008.155713

Mejia-Gervacio, S., Murray, K., and Lledo, P. M. (2011). NKCC1 controls GABAergic signaling and neuroblast migration in the postnatal forebrain. *Neural Dev.* 6, 4. doi: 10.1186/1749-8104-6-4

Merz, K., Herold, S., and Lie, D. C. (2011). CREB in adult neurogenesis–master and partner in the development of adult-born neurons? *Eur. J. Neurosci.* 33, 1078–1086. doi: 10.1111/j.1460-9568.2011.07606.x

Nguyen, L., Malgrange, B., Breuskin, I., Bettendorff, L., Moonen, G., Belachew, S., et al. (2003). Autocrine/paracrine activation of the GABA(A) receptor inhibits the proliferation of neurogenic polysialylated neural cell adhesion molecule-positive (PSA-NCAM+) precursor cells from postnatal striatum. *J. Neurosci.* 23, 3278–3294.

Nissant, A., Bardy, C., Katagiri, H., Murray, K., and Lledo, P. M. (2009). Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat. Neurosci.* 12, 728–730. doi: 10.1038/nn.2298

Overstreet Wadiche, L., Bromberg, D. A., Bensen, A. L., and Westbrook, G. L. (2005). GABAergic signaling to newborn neurons in dentate gyrus. *J. Neurophysiol.* 94, 4528–4532. doi: 10.1152/jn.00633.2005

Pallotto, M., Nissant, A., Fritschy, J. M., Rudolph, U., Sassoe-Pognetto, M., Panzanelli, P., et al. (2012). Early formation of GABAergic synapses governs the development of adult-born neurons in the olfactory bulb. *J. Neurosci.* 32, 9103–9115. doi: 10.1523/JNEUROSCI.0214-12.2012

Panzanelli, P., Bardy, C., Nissant, A., Pallotto, M., Sassoe-Pognetto, M., Lledo, P. M., et al. (2009). Early synapse formation in developing interneurons of the adult olfactory bulb. *J. Neurosci.* 29, 15039–15052. doi: 10.1523/JNEUROSCI.3034-09.2009

Panzanelli, P., Fritschy, J. M., Yanagawa, Y., Obata, K., and Sassoe-Pognetto, M. (2007). GABAergic phenotype of periglomerular cells in the rodent olfactory bulb. *J. Comp. Neurol.* 502, 990–1002. doi: 10.1002/cne.21356

Panzanelli, P., Gunn, B. G., Schlatter, M. C., Benke, D., Tyagarajan, S. K., Scheiffele, P., et al. (2011). Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J. Physiol.* 589(Pt 20), 4959–4980. doi: 10.1113/jphysiol.2011.216028

Pathania, M., Yan, L. D., and Bordey, A. (2010). A symphony of signals conducts early and late stages of adult neurogenesis. *Neuropharmacology* 58, 865–876. doi: 10.1016/j.neuropharm.2010.01.010

Patrizi, A., Scelfo, B., Viltono, L., Briatore, F., Fukaya, M., Watanabe, M., et al. (2008). Synapse formation and clustering of neuroligin-2 in the absence of GABAA receptors. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13151–13156. doi: 10.1073/pnas.0802390105

Petreanu, L., and Alvarez-Buylla, A. (2002). Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J. Neurosci.* 22, 6106–6113.

Platel, J. C., and Kelsch, W. (2013). Role of NMDA receptors in adult neurogenesis: an ontogenetic (re)view on activity-dependent development. *Cell. Mol. Life Sci.* 70, 3591–3601. doi: 10.1007/s00018-013-1262-z

Poulopoulos, A., Aramuni, G., Meyer, G., Soykan, T., Hoon, M., Papadopoulos, T., et al. (2009). Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. *Neuron* 63, 628–642. doi: 10.1016/j.neuron.2009.08.023

Rakic, P. (2002). Adult neurogenesis in mammals: an identity crisis. *J. Neurosci.* 22, 614–618.

Saiepour, L., Fuchs, C., Patrizi, A., Sassoe-Pognetto, M., Harvey, R. J., and Harvey, K. (2010). Complex role of collybistin and gephyrin in GABAA receptor clustering. *J. Biol. Chem.* 285, 29623–29631. doi: 10.1074/jbc.M110.121368

Saino-Saito, S., Cave, J. W., Akiba, Y., Sasaki, H., Goto, K., Kobayashi, K., et al. (2007). ER81 and CaMKIV identify anatomically and phenotypically defined subsets of mouse olfactory bulb interneurons. *J. Comp. Neurol.* 502, 485–496. doi: 10.1002/cne.21293

Shao, Z., Puche, A. C., Kiyokage, E., Szabo, G., and Shipley, M. T. (2009). Two GABAergic intraglomerular circuits differentially regulate tonic and phasic presynaptic inhibition of olfactory nerve terminals. *J. Neurophysiol.* 101, 1988–2001. doi: 10.1152/jn.91116.2008

Sieghart, W., Fuchs, K., Tretter, V., Ebert, V., Jechlinger, M., Hoger, H., et al. (1999). Structure and subunit composition of GABA(A) receptors. *Neurochem. Int.* 34, 379–385. doi: 10.1016/S0197-0186(99)00045-5

Sieghart, W., and Sperk, G. (2002). Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr. Top. Med. Chem.* 2, 795–816. doi: 10.2174/1568026023393507

Song, J., Sun, J., Moss, J., Wen, Z., Sun, G. J., Hsu, D., et al. (2013). Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. *Nat. Neurosci.* 16, 1728–1730. doi: 10.1038/nn.3572

Song, J., Zhong, C., Bonaguidi, M. A., Sun, G. J., Hsu, D., Gu, Y., et al. (2012a). Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature* 489, 150–154. doi: 10.1038/nature11306

Song, N. Y., Shi, H. B., Li, C. Y., and Yin, S. K. (2012b). Interaction between taurine and GABA(A)/glycine receptors in neurons of the rat anteroventral cochlear nucleus. *Brain Res.* 1472:1–10. doi: 10.1016/j.brainres.2012.07.001

Stewart, R. R., Hoge, G. J., Zigova, T., and Luskin, M. B. (2002). Neural progenitor cells of the neonatal rat anterior subventricular zone express functional GABA(A) receptors. *J. Neurobiol.* 50, 305–322. doi: 10.1002/neu.10038

Studler, B., Sidler, C., and Fritschy, J. M. (2005). Differential regulation of GABA(A) receptor and gephyrin postsynaptic clustering in immature hippocampal neuronal cultures. *J. Comp. Neurol.* 484. 344–355. doi: 10.1002/cne.20472

Sun, C., Sieghart, W., and Kapur, J. (2004). Distribution of alpha1, alpha4, gamma2, and delta subunits of GABAA receptors in hippocampal granule cells. *Brain Res.* 1029, 207–216. doi: 10.1016/j.brainres.2004.09.056

Toni, N., Teng, E. M., Bushong, E. A., Aimone, J. B., Zhao, C., Consiglio, A., et al. (2007). Synapse formation on neurons born in the adult hippocampus. *Nat. Neurosci.* 10, 727–734. doi: 10.1038/nn1908

Tozuka, Y., Fukuda, S., Namba, T., Seki, T., and Hisatsune, T. (2005). GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* 47, 803–815. doi: 10.1016/j.neuron.2005.08.023

Tyagarajan, S. K., and Fritschy, J. M. (2010). GABA(A) receptors, gephyrin and homeostatic synaptic plasticity. *J. Physiol.* 588(Pt 1), 101–106. doi: 10.1113/jphysiol.2009.178517

Tyagarajan, S. K., Ghosh, H., Harvey, K., and Fritschy, J. M. (2011). Collybistin splice variants differentially interact with gephyrin and Cdc42 to regulate gephyrin clustering at GABAergic synapses. *J. Cell Sci.* 124(Pt 16), 2786–2796. doi: 10.1242/jcs.086199

Tyagarajan, S. K., Ghosh, H., Yevenes, G. E., Imanishi, S. Y., Zeilhofer, H. U., Gerrits, B., et al. (2013). Extracellular signal-regulated kinase and glycogen synthase kinase 3beta regulate gephyrin postsynaptic aggregation and GABAergic synaptic function in a calpain-dependent mechanism. *J. Biol. Chem.* 288, 9634–9647. doi: 10.1074/jbc.M112.442616

Vadodaria, K. C., and Jessberger, S. (2013). Maturation and integration of adult born hippocampal neurons: signal convergence onto small Rho GTPases. *Front. Synaptic Neurosci.* 5:4. doi: 10.3389/fnsyn.2013.00004

van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034. doi: 10.1038/4151030a

Waclaw, R. R., Allen, Z. J. 2nd., Bell, S. M., Erdelyi, F., Szabo, G., Potter, S. S., et al. (2006). The zinc finger transcription factor Sp8 regulates the generation and diversity of olfactory bulb interneurons. *Neuron* 49, 503–516. doi: 10.1016/j.neuron.2006.01.018

Wang, D. D., and Kriegstein, A. R. (2009). Defining the role of GABA in cortical development. *J. physiol.* 587, 1873–1879. doi: 10.1113/jphysiol.2008.167635

Wang, D. D., Krueger, D. D., and Bordey, A. (2003). GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAA receptor activation. *J. Physiol.* 550(Pt 3), 785–800. doi: 10.1113/jphysiol.2003.042572

Wei, W., Zhang, N., Peng, Z., Houser, C. R., and Mody, I. (2003). Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J. Neurosci.* 23, 10650–10661.

Whitman, M. C., and Greer, C. A. (2007). Synaptic integration of adult-generated olfactory bulb granule cells: basal axodendritic centrifugal input precedes apical dendrodendritic local circuits. *J. Neurosci.* 27, 9951–9961. doi: 10.1523/JNEUROSCI.1633-07.2007

Winner, B., Cooper-Kuhn, C. M., Aigner, R., Winkler, J., and Kuhn, H. G. (2002). Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur. J. Neurosci.* 16, 1681–1689. doi: 10.1046/j.1460-9568.2002.02238.x

Young, S. Z., Lafourcade, C. A., Platel, J. C., Lin, T. V., and Bordey, A. (2014). GABAergic striatal neurons project dendrites and axons into the postnatal subventricular zone leading to calcium activity. *Front. Cell. Neurosci.* 8:10. doi: 10.3389/fncel.2014.00010

Young, S. Z., Platel, J. C., Nielsen, J. V., Jensen, N. A., and Bordey, A. (2010). GABA(A) increases calcium in subventricular zone astrocyte-like cells through L- and T-Type voltage-gated calcium channels. *Front. Cell. Neurosci.* 4:8. doi: 10.3389/fncel.2010.00008

Yuste, R., and Katz, L. C. (1991). Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron* 6, 333–344. doi: 10.1016/0896-6273(91)90243-S

Zhao, S., Zhou, Y., Gross, J., Miao, P., Qiu, L., Wang, D., et al. (2010). Fluorescent labeling of newborn dentate granule cells in GAD67-GFP transgenic mice: a genetic tool for the study of adult neurogenesis. *PLoS ONE* 5:e12506. doi: 10.1371/journal.pone.0012506

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